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Association between the luteinizing hormone/chorionic gonadotropin receptor (*LHCGR*) rs4073366 polymorphism and ovarian hyperstimulation syndrome during controlled ovarian hyperstimulation

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Abstract

Background: The aim of this study was to determine the relationship between a purported luteinizing hormone/chorionic gonadotropin (*LHCGR*) high function polymorphism (rs4539842/*ins*LQ) and outcome to controlled ovarian hyperstimulation (COH).

Methods: This was a prospective study of 172 patients undergoing COH at the Fertility and IVF Center at GWU. DNA was isolated from blood samples and a region encompassing the *ins*LQ polymorphism was sequenced. We also investigated a polymorphism (rs4073366 G > C) that was 142 bp from *ins*LQ. The association of the *ins*LQ and rs4073366 alleles and outcome to COH (number of mature follicles, estradiol level on day of human chorionic gonadotropin (hCG) administration, the number of eggs retrieved and ovarian hyperstimulation syndrome (OHSS)) was determined.

Results: Increasing age and higher day 3 (basal) FSH levels were significantly associated with poorer response to COH. We found that both *ins*LQ and rs4073366 were in linkage disequilibrium (LD) and no patients were homozygous for both recessive alleles (*ins*LQ/*ins*LQ; C/C). The *ins*LQ variant was not significantly associated with any of the main outcomes to COH. Carrier status for the rs4073366 C variant was associated ($P = 0.033$) with an increased risk (OR 2.95, 95% CI = 1.09-7.96) of developing OHSS.

Conclusions: While age and day 3 FSH levels were predictive of outcome, we found no association between *ins*LQ and patient response to COH. Interestingly, rs4073366 C variant carrier status was associated with OHSS risk. To the best of our knowledge, this is the first report suggesting that *LHCGR* genetic variation might function in patient risk for OHSS.

Keywords: Ovarian stimulation, Ovarian hyperstimulation syndrome, OHSS, LHCGR, LHR, Polymorphism

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Background

Controlled ovulation hyperstimulation (COH) is the cornerstone of assisted reproduction. The use of exogenous gonadotropins subverts the natural process of single follicular dominance and allows for the recruitment and maturation of multiple ova during the ovarian cycle. Although this technique has vastly enhanced the potential for *in vitro* fertilization (IVF) success, individual patients still have disparate responses. Various phenotypic predictors of ovarian responsiveness (i.e. ovarian reserve testing (ORT)) [1-4] have been used to titrate doses of fertility medications. Despite such predictors, gonadotropin dosing remains somewhat empiric and thus, patients risk under or over responding (ovarian hyperstimulation syndrome (OHSS)) to these drugs. Pharmacogenetic biomarkers offer promise for aiding in the *a priori* determination of patient response to COH [5] and minimizing complications. To date, most work has focused on common variant alleles of follicle stimulating hormone receptor (*FSHR*) [6-17], estrogen receptor (*ESR*) [6-8,18-20] and aromatase (*CYP19A1*) [6,21] genes as well as several other genetic loci [8,10,22,23] which have offered some promising predictive biomarkers for COH outcome.

The luteinizing hormone receptor (LHR) protein is a G protein-coupled hormone receptor (GPCR) [24] and is expressed in numerous tissues including the gonads [25,26], uterus [26-28], fallopian tubes [29], placenta and fetus [30]. Both LH and human chorionic gonadotropin (hCG) are endogenous ligands for LHR [31] with the latter also employed during COH. Prior to ovulation, FSH and estradiol both increase pituitary production of LH and induce LHR expression in the ovaries where LHR functions in promoting follicular maturation, lutenization and ovulation [32]. During assisted reproduction, it is believed that hCG administration (i.e. hCG trigger) activates LHR-mediated signaling. The pharmacological use of hCG for COH has been implicated in the increased ovarian vascular permeability associated with OHSS, an iatrogenic complication of COH [33-36].

LHR is encoded by the luteinizing hormone/chorionic gonadotropin receptor (*LHCGR*) gene (~69 kb) located on chromosome 2p21 [37-39]. *LHCGR* harbors at least 300 known polymorphisms [40-42] some of which having a significant impact on sexual development and fertility [43-51]. Recently, a 6 base-pair (CTCCAG) insertion in exon 1 (rs4539842; *ins*LQ) has been reported that results in the addition of two amino acids (Leu-Gln) in the signal peptide region of the receptor [42,52-54]. The allelic frequency of the *ins*LQ polymorphism is approximately 0.3 in individuals of Caucasian and African descent [54,55]. Structurally, *ins*LQ impacts LHR by potentially altering protein folding, trafficking and membrane insertion. Functionally, the *ins*LQ variant LHR protein displays higher activity in cell culture potentially due to improved trafficking and increased cell surface expression, but unrelated to alterations in hCG binding [52]. Breast cancer patients carrying the *ins*LQ allele exhibit shorter disease-free survival than non-carriers [52,53]. In addition, *LHCGR* resides near a potential susceptibility locus for polycystic ovary syndrome (PCOS), which is characterized by anovulation/oligo-ovulation and elevated androgen levels [56]. In a small case/control study (n = 72), *ins*LQ was detected at a higher level (40.5%) in patients who experienced ovarian hyperstimulation syndrome (OHSS) compared to controls (27.5%), but this did not achieve statistical significance potentially due to a small sample size [57].

Patient response to COH is multi-factorial in nature. To date, there is no reliable test or algorithm that combines both genetic and clinical factors for predicting patient response to fertility therapy. As a result, the focus of this ongoing work is to identify genetic factors that are predictive of clinical response to COH. The *ins*LQ allele appears to result in an LHR protein with increased *in vitro* activity; it is therefore possible that this polymorphism could significantly impact patient response to COH. The objective of this study was to investigate the relationship between the *LHCGR ins*LQ and clinical response to COH. Interestingly, the results suggest that *ins*LQ has little impact on COH outcome. However, carriers of a single nucleotide polymorphism (rs4073366 G > C) nearby *ins*LQ exhibited an increased risk of developing OHSS.

Methods

This study was approved by George Washington University Institutional Review Board. Written informed consent was obtained from the participants of this study. The study was open to all adult (>18 years of age), female patients seeking treatment at the GW Fertility and IVF Center at GWU Medical Center. One hundred seventy-two patients were recruited into the study from 2010–2011. All IVF patients being monitored while taking injectable gonadotropins were invited to participate. Prior to beginning treatment, all patients had undergone an evaluation that included ovarian reserve testing, semen analysis (male partner), uterine cavity study and thyroid screening. Controlled ovarian stimulation was accomplished with either luteal down regulation using a GnRH agonist (leuprolide acetate; TAP Pharmaceuticals) followed by recombinant FSH (Follistim; Merck & Co; or Gonal-F, Serono) administration or with recombinant FSH (Follistim; Merck & Co; or Gonal-F, Serono) administration in combination with a GnRH antagonist (Antagon; Merck & Co). The initial FSH dose was dependent upon patient age and basal ovarian reserve testing. After initial follicular monitoring (serum estradiol and transvaginal ultrasound assessments), FSH dosing was titrated based upon the ovarian response. hCG trigger was withheld for levels E2 over 4000 pg/ml thus minimizing risk for OHSS.

Both control and OHSS groups had similar risk factors including those identified at time of hCG trigger. Ovarian response to gonadotropin was observed. The primary endpoint of the study was the estradiol level on day of hCG injection. Secondary clinical endpoints included number of ovarian follicles counted on day of hCG number of eggs retrieved and the incidence of OHSS. Ovarian hyperstimulation syndrome was defined clinically based on the criteria established by Navot [58,59].

Approximately 5 mL of blood was collected for genetic analysis at the GW IVF Center. Total genomic DNA was extracted from 200 μl EDTA anti-coagulated venous blood using a QiaCube automated instrument with the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). The samples were frozen at −80°C until the time of genotyping. A 291 base-pair target region encompassing (rs4539842/*ins*LQ and rs4073366 was amplified using 10 pmol/μl forward (5'-CACTCAGAGGCCGTCCAAG-3') and reverse (5'-GGAGGGAAGGTGGCATAGAG-3') primers [42]. PCR was performed in a reaction volume of 50 μl, including 10.0 μl of 5X Reaction Buffer, 0.5 μl Go*Taq* L nucleotide mix, 6 μl $MgCl_2$ DNA Polymerase (Promega Corp, Madison, WI), 3.75 μl of H_2O, and 10.0 μl of genomic DNA. The PCR cycling conditions were as follows: 1 cycle of 95°C for 2 minutes, followed by 32 cycles of 95°C for 30 seconds, 56°C for 30 seconds and 72°C for 30 seconds and a 7 min final extension at 72°C. PCR products were purified (GeneClean Turbo, MP Biomedicals, Solon, OH) and 20–40 ng/μl of samples were sequenced using BigDye Terminator technology (Life Technologies, Grand Island, NY) by Eurofins MWG Operon (Huntsville, AL). Multiple sequence alignments were carried out using ChromasPro software (Technelysium Pty Ltd).

Patient differences in mean outcomes (estradiol level, number of follicles, number of eggs retrieved) related to genotype were tested using a step-down bootstrap resampling method (the STEPBOOT option in PROC MULTTEST in SAS) [60]. This method adjusts for multiple comparisons while maintaining relatively good statistical power compared to other methods and is applicable to both normal and non-normally distributed data [60]. Outcome differences were first tested separately for each polymorphism, followed by comparing combinations of alleles for the two polymorphisms. The association between the distributions of patients across alleles for the two polymorphisms was tested by Fisher's Exact Test. Multivariable model predicting each outcome was calculated using linear regression for estradiol level and negative-binomial regression for number of follicles and eggs retrieved. The latter method is an extension of Poisson regression. SAS v.9.2 (SAS Institute, Cary, NC) was used for all statistical analyses. Polymorphisms and were analyzed for Hardy-Weinberg equilibrium and linkage disequilibrium (LD) using PyPop: Python of Population Genomics software [61], CubeX [62] and SPSS (IBM, Armonk, NY). Unadjusted odds ratios for OHSS risk were determined using SAS v.9.2 and SPSS.

Results

A total of 172 patients underwent IVF and were genotyped. The mean age of the patient population was 36.8 years old (Table 1). The majority of the population was Caucasian (57.0%) followed by Asian/Pacific Islander (15.7%), non-Hispanic, African American (11.1%) and Hispanic (2%). Because there were few Hispanic patients, for analysis we grouped these individuals with those for which no self-identified ethnic/ancestral information was available (16.3% of sample). Median levels of ovarian reserve markers TSH, day 3 follicle-stimulating hormone (FSH) and day 3 estradiol (E_2) levels, were 1.8 IU/ml, 7.0 IU/L, and 40.8 pg/ml, respectively. On average, patient gonadotropin stimulation lasted 12 days. Mean E_2 levels on the day of hCG administration was 1780 pg/ml and the median number of follicles and eggs retrieved was 10 and 9, respectively. Eighteen patients (10.5%) experienced moderate to severe OHSS (Table 1). The OHSS cases in our sample were similar to the 154 non-OHSS patients on age and most relevant clinical variables, with the exception of being more likely to be Black, less likely to be Asian. OHSS cases also had higher numbers of follicles, a higher mean estradiol level on day of hCG and more eggs retrieved (Table 1). These three clinical differences were expected based on the nature of OHSS.

Recently, a novel, potentially inactivating, mutation was detected that resided within the *ins*LQ insertion (CTGCA > CG) in a patient with poor oocyte recovery following IVF [44]. This mutation would not have been identified using fragment analysis (the commonly employed method for detecting *ins*LQ). As a result, we analyzed *ins*LQ through direct sequencing of a 291 base-pair region of exon/intron 1. As shown in Table 2, relative allelic proportions for no-*ins*LQ/no-*ins*LQ, no-*ins*LQ/*ins*LQ and *ins*LQ/*ins*LQ were ~0.62, 0.34 and 0.035, respectively (n = 172). Another polymorphism, rs4073366 G > C, occurs ~142 bp downstream of *ins*LQ and was detected during sequencing. We attempted alternative methods (TaqMan® probe, alternative primer pairs) to omit rs4073366 from our study of *ins*LQ, but these yielded unreliable results. Consequently, we included this single nucleotide polymorphism (SNP) in our analysis because of its potential impact on any apparent associations between *ins*LQ and COH outcome. For rs4073366, G/G, G/C and C/C variants occurred at relative proportions of ~0.72, 0.25 and 0.03, respectively in our patient population. Both variants were in Hardy-Weinberg equilibrium (HWE) and *ins*LQ and rs4073366 were in significant LD (D' = 1.0, $P < 0.0001$) as determined by pair-wise LD estimation (Table 2) (49). Homozygosity or heterozygosity for

Table 1 Demographic, clinical, and genetic characteristics of the study sample (N = 172)

	Total	OHSS Cases	Controls
Mean age (SD), Years	36.8 (4.1)	35.2 (2.9)	37 (4.2)
*Race**	–	–	–
White non-Hispanic	98 (57.0)	12 (66.7)	86 (55.8)
Black, non-Hispanic	19 (11.1)	5 (27.8)	14 (9.1)
Asian/Pacific Islander	27 (15.7)	1 (5.6)	26 (16.9)
Hispanic/Missing	28 (16.3)	0 (0.0)	28 (18.2)
*TSH (IU/mL)***	1.8 (1.2-2.5)	1.8 (1.5-2.1)	1.8 (1.2-2.6)
*Day 3 FSH level (IU/L)***	7.0 (5.4-8.5)	6.3 (4.5-7.3)	7.0 (5.5-8.5)
*Day 3 Estradiol level (pg/mL)***	40.8 (30.0-55.7)	41.6 (33.3-57.8)	39.0 (30.0-54.7)
*Total number of follicles***	10.0 (6.0-15)	18.0 (13.0-24.0)	9.0 (5.0-12.0)
*Total number of days stimulated **	12 (10–13)	12 (11–13)	12 (10–13)
*Estradiol level day of hCG **	1780 (1136–2401)	2606 (1953–3436)	1735 (988–2254)
*Total number of eggs retrieved **	9 (5–14)	18 (13–23)	8 (4–13)

* All categorical variables are presented as N (%).
** Presented as median (IQR) due to highly skewed distributions.

*ins*LQ was associated with not being CG or CC for rs4073366 (Table 3, $P = 0.012$). In addition, we did not find any individuals who were homozygous for both *ins*LQ and rs4073366, nor did we identify any patients who had the *ins*LQ, rs4073366 "C" haplotype (data not shown).

Of the 172 patients, up to 17 patients were excluded (depending on the clinical endpoint) from multivariable analysis because of missing data values related to being screened at outside centers that did not have complete ovarian reserve testing or they did not undergo follicular monitoring on day of hCG administration. Both age and ORT (day 3 FSH, TSH and E2 levels) have been prognostic clinical indicators of outcome to COH (52). As a result, regression models predicting estradiol level on day of HCG, number of follicles, and number of eggs retrieved included age, day 3 FSH level, day 3 estradiol levels, and the *ins*LQ and rs4073366 polymorphisms as covariates. In the multivariable models (Table 4), patient age was significantly associated with lower E2 levels on day of hCG ($P = 0.03$), fewer number of follicles ($P < 0.0001$) and a lower number of eggs retrieved ($P = 0.0002$). Increasing basal FSH level was associated with fewer follicles ($P = 0.009$) and eggs retrieved ($P = 0.0002$), but not E2 levels ($P = 0.46$) on day of hCG. All three main outcomes were positively correlated with each other (Spearman r, $P < 0.0001$) (data not shown). Finally, self-identified race/ethnicity was not significantly associated with any of the three main patient outcome measures (data not shown).

None of the pairwise differences comparing estradiol levels, number of follicles, and number of eggs retrieved among the 3 *ins*LQ genotypes reached statistical significance (not shown). Similarly, results for rs4073366 genotype were also not significant for these three outcomes (not shown). We next examined the 6 non-zero combination genotypic groups and, again, found no significant differences for any of the outcomes when each mean was compared to the overall mean (Table 5). However, there was a non-significant trend for those individuals who did not carry *ins*LQ and were heterozygous (GC) for rs4073366 (i.e. no-*ins*LQ/no-*ins*LQ + CG) to have increased mean estradiol level on day of hCG compared to the mean across all other groups ($P = 0.10$). The *ins*LQ

Table 2 Allelic frequencies and linkage disequilibrium for the rs4539842 (insLQ) and rs4073366 polymorphisms

Variant	Frequency (n = 172)	D′	r^2	LD *P*-value[a]
rs4539842 (insLQ)	–	–	–	–
no-insLQ/no-insLQ	0.622 (107)	–	–	–
no-insLQ/insLQ	0.340 (59)	–	–	–
insLQ/insLQ	0.035 (6)	–	–	–
		–	–	–
rs4073366	–	–	–	–
GG	0.721 (124)	–	–	–
GC	0.250 (43)	–	–	–
CC	0.029 (5)	–	–	–
insLQ /rs4073366	–	1.00	0.0474	<0.0001

[a] Both variants were in Hardy-Weinberg equilibrium (HWE).

Table 3 Frequency of *ins*LQ and rs4073366 (N = 172)*

	no-insLQ/no-insLQ	no-insLQ/ *ins*LQ	*ins*LQ/*ins*LQ
GG	68 (39.5%)	50 (29.1%)	6 (3.5%)
CG	34 (19.8%)	9 (5.2%)	0
CC	5 (2.9%)	0	0

*n (%), Fisher's exact test for association between *ins*LQ and rs4073366: $P = 0.012$.

Table 4 Adjusted regression of demographic, clinical and genetic predictors of estradiol level on day of hCG, follicle count, and egg count*

Factor	Estradiol Level on Day of hCG (N = 157)[a]			Number of Follicles (N = 155)[b]			Number of Eggs Retrieved (N = 160)[c]		
	Estimate	95% CI	P-Value	Estimate	95% CI	P-Value	Estimate	95% CI	P-Value
Age	−48.2	−90.3, -6.1	0.03	0.939	0.918, 0.961	<0.0001	0.951	0.927, 0.977	0.0002
Day 3 FSH level	−10.3	−38.0, 17.3	0.46	0.976	0.958, 0.994	0.0097	0.953	0.930, 0.977	0.0002
Day 3 Estradiol level	−2.5	−7.7, 2.7	0.34	0.999	.997, 1.002	0.57	0.998	0.995, 1.000	0.08
insLQ/insLQ, GG	5.9	−818.3, 830.2	0.98	0.963	0.617, 1.503	0.32	0.647	0.380, 1.111	0.11
no-insLQ/insLQ, GG	−197.2	−538.9, 144.4	0.26	0.909	0.755, 1.096	0.87	0.952	0.772, 1.174	0.64
no-insLQ/no-insLQ, CC	−784.1	−1,682.0, 113.7	0.09	0.903	0.557, 1.63	0.68	0.665	0.372, 1.189	0.17
no-insLQ/no-insLQ, CG	163.8	−224.3, 551.9	0.41	1.210	0.985, 1.488	0.07	1.211	0.963, 1.523	0.10

* Estimates adjusted for all other variables presented.
[a] Linear regression with estimates of change in mean estradiol levels (pg/ml).
[b] Negative binomial regression with estimates of relative odds of number of follicles.
[c] Negative binomial regression with estimates of relative odds of number of eggs retrieved.

polymorphism (and rs4073366) was not associated with IVF protocol type, age, or basal FSH or E2 levels (data not shown).

The *ins*LQ variant is purportedly a high-function allele and, accordingly, might place patients at a higher risk of OHSS. As a result, we tested whether either *ins*LQ or rs4073366 were associated with an increased risk of OHSS. Because of the small number of OHSS patients, logistic regression analyses were performed without adjustment for covariates. Intriguingly, the *ins*LQ variant was not associated with OHSS by either genotype ($P = 0.788$) (data not shown) or *ins*LQ carrier status (Table 6). There was a non-significant (P = 0.07) trend towards an association between rs4073366 genotype and OHSS. When rs4073366 carrier status was included in our analysis, carriers of the rs4073366 C allele exhibited a significantly (P = 0.033) higher risk of OHSS (OR = 2.95, 95% CI = 1.09-7.96). Further haplotype analysis revealed that only the no-*ins*LQ/C haplotype was significant ($P = 0.023$) for OHSS risk (OR = 2.46, 95% CI 1.11-5.46) (data not shown).

Discussion

LHR-mediated signaling plays an important role in patient response to exogenous gonadotropins (i.e. hCG) administered during COH and inter-individual variability in LHR activity could significantly impact outcome. As a result, we investigated whether *LHCGR* genetic variation influences response to COH. We focused our analysis on the *ins*LQ polymorphism (rs4539842) and the rs4073366 G > C SNP located downstream of *ins*LQ in intron 1. We found that insLQ was not associated with patient response to COH, nor was it a predictor for OHSS. Therefore, it is possible that the improved function conferred to LHR by this polymorphism *in vitro* [52] is not reflective of the situation in the ovaries. Moreover, *ins*LQ activity was previously investigated in HEK-293 cells which may not accurately replicate the behavior of the *ins*LQ receptor variant ovarian granulosa cells [52]. In contrast, we found that carriers of the rs4073366 "C" allele exhibited a ~3-fold increased risk of developing OHSS.

Very little information exists regarding polymorphisms that are predictive of patients developing OHSS. For example, the *FSHR* Thr307Ala polymorphism has been linked with iatrogenic OHSS in an Indian population [63], but not in European [9] or Brazilian patients [64]. The well-studied Asn680Ser *FSHR* polymorphism has not been associated with OHSS, but it is potentially predictive of the severity of symptoms [9]. In addition, *BMP15* variation, such as the rs3810682 SNP (OR = 2.7, 95% CI = 1.3-5.7), has also been implicated in OHSS [23,65]. Finally, *VEGFA* gene variation was recently identified as a risk allele (OR = 3.4, 95% CI = 1.01-11.7) for OHSS [66]. Given the paucity of genetic risk factors for OHSS, one of the most significant findings of this work

Table 5 Mean (SD) of estradiol level, number of follicles and number of eggs retrieved by *ins*LQ and rs4073366±

	Number of patients	Estradiol level	Number of follicles	Number of eggs retrieved
no-insLQ/no-insLQ + GG	64-66	1819.5 (986.3)	9.8 (5.7)	9.3 (6.7)
no-insLQ/ insLQ + GG	48-49	1922.4 (1033.5)	10.9 (6.7)	11.4 (8.8)
insLQ/insLQ + GG	6	1946.0 (860.6)	10.3 (8.9)	6.7 (6.3)
no-insLQ/no-insLQ + CG	32-34	2156.1 (1009.4.0)*	13.5 (7.4)	13.3 (7.9)
no-insLQ/ insLQ + CG	8-9	1232.3 (700.3)	7.8 (3.7)	9.4 (3.7)
no-insLQ/no-insLQ + CC	5	1201.6 (464.9)	10.4 (7.4)	7.2 (0.83)

* $P = 0.10$.

Table 6 Analysis of *ins*LQ/ rs4539842 and rs4073366 carrier status and OHSS risk

Variant	OHSS Cases (%)	Controls (%)	OR (95% CI)[#]	P-value*
***ins*LQ/rs4073366**				
Non-Carrier	12 (66.7)	95 (61.7)		
Carrier	6 (33.3)	59 (38.3)	0.81 (0.29-2.26)	0.6807
rs4539842 (C Allele)				
Non-carrier	9 (50.0)	115 (74.7)		
Carrier	9 (50.0)	39 (25.3)	2.95 (1.09-7.96)	0.0328

[#] Unadjusted OR.
* Logistic regression.

was the association of rs4073366 C allele carrier status with increased risk of OHSS (OR = 2.95, 95% CI 1.09-7.96, Table 6) during COH. Furthermore, it seems that the effect of rs4073366 on OHSS risk was largely related to the no-insLQ/C haplotype (OR = 2.46, 95% CI 1.11-5.46) (data not shown). This interesting finding requires further investigation in other COH populations and suggests that the LHCGR genetic variation influences OHSS development. A recent genome-wide association study found that *LHCGR* was associated with serum steroid hormone binding globulin (SHBGs) levels (62), which could result in elevated serum concentrations of androgens and estrogen. However, the impact of LHCGR polymorphisms (i.e. *ins*LQ, rs4073366) on SHBG levels in not currently known.

While rs4073366 is a potential predictor of OHSS risk, the functional consequences of this polymorphism on LHR function are yet to be elucidated. rs4073366 has a major allele of "C" on the "+" strand ("G" on the "-"strand in this study) and resides in a cryptic 3' splice acceptor site (data not shown) which could potentially impact *LHCGR* mRNA processing yielding a splice variant with altered activity [67]. In addition, the intronic region surrounding rs4073366 is complementary to *APOE* mRNA and has been associated with decreased risk of Alzheimer's disease (AD) in males carrying the *APOE* ε4 allele [67]. Given that apolipoprotein E is important for cholesterol uptake and steroidogenesis, and genetic variation in *APOE* has been linked to reproductive efficiency [68-70], it is possible that rs4073366 may alter response to fertility drugs via modulation of *APOE* mRNA stability. Although beyond the scope of this investigation; future work is focused on investigating the molecular consequences of rs4073366 on LHCGR function.

Conclusions

Outcome to COH is multi-factorial, variable and unpredictable. There have been few studies that have investigated *LHCGR* variability and its influence on COH. Here, we provide the first report of an association between *LHCGR* genetic variability and OHSS risk. The relevance of the rs4073366 polymorphism OHSS should be evaluated in additional patient populations. In addition, because OHSS is multigenic in nature, future work is warranted to investigate whether rs4073366, and other *LHCGR* variants, genetically interact with other loci to predict patient response to COH.

Abbreviations

COH: Controlled ovarian hyperstimulation; OHSS: Ovarian hyperstimulation syndrome; SNP: Single nucleotide polymorphism.

Competing interests

The authors declare that they have no competing interests associated with this work.

Authors' contributions

TO: Conceived of the study, participated in its design, carried out molecular analyses, assisted in data analysis and drafted the manuscript. MK: Conducted statistical analyses, assisted with drafting the manuscript. AH: Participated in study design, assisted in data analysis and drafted the manuscript. AC: Carried out molecular analysis with TO. IG: Processed samples, carried out molecular analysis with TO. SS: Worked with MK on all statistical analyses. DF: Conceived of the study, participated in its design, recruited participants, assisted with drafting the manuscript. PG: Conceived of the study, participated in its design, recruited participants, assisted with drafting the manuscript. All authors read and approved the final manuscript.

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