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ABSTRACT: Candidate-gene association studies that examined the association between polymorphisms of the inhibin alpha gene (G769A, C16T, and A124G) and premature ovarian failure (POF) have reported contradictory results. Thus, a meta-analysis of these studies was carried out. The random effects odds ratio (OR) with the corresponding 95% confidence interval and the heterogeneity among studies were estimated. Existence of potential bias and consistency of effect sizes across ethnicities were explored. Cumulative meta-analysis was also performed. The studies provided 1030/1660, 936/1398, and 938/1446 cases/controls for G769A, C16T, and A124G polymorphisms, respectively. The meta-analysis showed significant heterogeneity among the studies (PQ = 0.01, I^2 = 74%) and lack of evidence that carriers of the G769A variant confer risk of POF: OR = 1.38 (0.48–3.94). Asian Indians (only two studies) produced significant association [OR = 8.10 (1.27–51.6)]. Regarding C16T and A124G polymorphisms, 16T and 124G alleles were not associated with POF: OR = 0.94 (0.76–1.16) and OR = 0.98 (0.86–1.11), respectively. The cumulative meta-analysis for G769A and C16T polymorphisms showed a trend in time towards non-significance for both polymorphisms. Cumulative meta-analysis indicated that more evidence is needed to draw safer conclusions regarding the effect sizes. There was no differential magnitude of effect in large versus small studies. In conclusion, there is no evidence of association between the studied polymorphisms and POF.

Key words: premature ovarian failure / inhibin alpha / gene / polymorphism / meta-analysis

Introduction

Premature ovarian failure (POF) is a syndrome characterized by ovarian failure before the age of 40 years. POF occurs in approximately 1% of the women under the age of 40 years and in 0.1% of the women below the age of 30 years (Coulam et al., 1986). Family studies have shown a strong genetic contribution to the pathogenesis of POF (Mattison et al., 1984; Vegetti et al., 1998). The inhibin alpha (INHA) gene which is located on chromosome 2 (2q33–q36) encodes the alpha subunit of inhibins A and B and is implicated in POF susceptibility (Woad et al., 2009). Inhibin A and B regulate the FSH levels and a defect in the inhibins’ secretion may cause an increase in FSH concentration resulting to follicle recruitment alterations and premature depletion of the ovarian follicle pool (Shelling et al., 2000). In vitro functional analysis (Chand et al., 2007) has provided evidence that INHA G769A variant may increase susceptibility to POF with impaired inhibin B bioactivity.

The candidate-gene association studies that examined the association between polymorphisms in the INHA gene and POF have provided controversial or inconclusive results, partly because each study involved few cases and few controls and, therefore, there was not enough information to demonstrate association. Furthermore, the interpretation is complicated by the fact that different populations and number of loci included in the analyses were used. In order to shed some light on these contradictory results, as well as to decrease the uncertainty of the effect size of estimated risk, a meta-analysis of all available studies related the INHA G769A, C16T and A124G polymorphisms and their associations with POF was carried out using previously described methodology (Zintzaras and Lau, 2008a). In addition, the heterogeneity between studies, the existence of potential bias and the consistency of genetic risk effects across ethnicities were explored. Cumulative and recursive cumulative meta-analysis were also performed (Lau et al., 1992).
Materials and Methods

Selection of studies

Studies were identified by searching PubMed database for relevant articles in English published before May 2009 using as a search criterion combinations of the following terms: ‘inhibin alpha’, ‘INHA’, ‘gene’, ‘polymorphism’, ‘premature ovarian failure’, ‘POF’, ‘primary ovarian insufficiency’, ‘premature menopause’, ‘primary ovarian failure’, ‘hypergonadotropic hypogonadism’, ‘gonadal dysgenesis’, ‘association’, ‘susceptibility’ and ‘risk’. The retrieved articles were read in their entirety to assess their appropriateness for inclusion in the meta-analysis. All references cited in the articles were also reviewed to identify additional published work not indexed by the PubMed database. Case reports, editorials and review articles were excluded.

Case–control studies that determined the genotype distribution or the allele frequency of INHA polymorphisms in cases with POF and in healthy controls, were eligible for inclusion in the meta-analysis. In studies with overlapping cases or controls, the most recent and/or the largest in size study with extractable data was included in the meta-analysis. The meta-analysis considered only polymorphisms investigated in more than two studies. Only studies in human subjects that have used validated genotyping methods were considered. Family-based studies and hypothesis-free association studies were not considered because of different design considerations (Zintzaras and Lau, 2008a).

Data extraction

From each study the following information was extracted: first author, journal, year of publication, ethnicity of study population, matching, genotyping method, and frequency of mutant allele or the number of mutant-carriers in cases and controls for each polymorphism. In addition, it was recorded whether the genotypic data were read blinded to the disease status.

Data synthesis

The meta-analysis examined the association between each INHA polymorphism and POF. For C16T and A124G polymorphisms the associations between the mutant alleles (16T and 124G, respectively) and POF were examined. For the G769A polymorphism, the association between carriers of the G769A variant and POF was examined because the 769A allele is very rare. The associations were indicated as a pooled odds ratio (OR) with the corresponding 95% confidence interval (CI). The heterogeneity between studies was tested using the Q-statistic (Cochran, 1954). If $P_Q < 0.10$ then heterogeneity was considered statistically significant. Heterogeneity was quantified with the $I^2$ metric, which is independent of the number of studies in the meta-analysis. $I^2$ was calculated for more than two studies. $I^2$ takes values between 0 and 100% with higher values denoting greater degree of heterogeneity (Zintzaras and Lau, 2008a). The pooled OR was estimated using random effects (RE) (DerSimonian and Laird, 1986) model. RE modeling assumes a genuine diversity in the results of various studies, and it incorporates to the calculations a between study variance. When there is lack of heterogeneity the RE model coincides with the fixed effects model (Trikalinos et al., 2008).

A cumulative and recursive cumulative meta-analysis was carried out in order to evaluate the trend of OR in time (Lau et al., 1992; Zintzaras and Lau, 2008a). In cumulative meta-analysis, studies were chronologically ordered by publication year, then, the pooled OR was obtained at the end of each year, i.e. at each information step. In recursive cumulative meta-analysis, the relative change in pooled OR in each information step was calculated. Cumulative and recursive cumulative meta-analysis, provide a frame work for updating a genetic effect from all studies and a measure of how much the genetic effect changes as evidence accumulates. Thus, cumulative meta-analysis indicates the trend in estimated risk effect and recursive cumulative meta-analysis indicates the stability in risk effect. A differential magnitude of effect in large versus small studies was checked using the Egger regression test (Egger et al., 1997).

The meta-analysis consisted of the main (overall) analysis which includes all available data, subgroup analyses by ethnicity and sensitivity analysis which examines the effect of excluding specific studies (Zintzaras, 2006). The genotype distribution of control group was tested for Hardy–Weinberg equilibrium (HWE) using an exact test (Weir, 1996). HWE is considered a surrogate of study quality (Zintzaras, 2006) and studies with controls not in HWE were subjected to a sensitivity analysis. Analyses were performed using CUMAGAS (http://biomath.med.uth.gr), and Compaq Visual Fortran90 with International Mathematics and Statistics Library.

Results

Eligible studies

The literature review identified 74 articles in PubMed that met the search criteria. The full articles and their references were analyzed to assess their appropriateness for meta-analysis. Data from nine articles met the inclusion criteria (Shelling et al., 2000; Marozzi et al., 2002; Jeong et al., 2004; Harris et al., 2005; Dixit et al., 2006; Sundblad et al., 2006; Corre et al., 2009; Prakash et al., 2009; Woad et al., 2009). However, in two studies (Marozzi et al., 2002; Corre et al., 2009) the data were overlapped, thus, the smallest study (Marozzi et al., 2002) was disregarded. Therefore, eight studies were included in the meta-analysis. One article (Corre et al., 2009) provided data on three separate study populations and they treated as different studies. The articles had been published between 2000 and 2009.

Eight studies provided data for carriers of the G769A variant (Shelling et al., 2000; Jeong et al., 2004; Dixit et al., 2006; Sundblad et al., 2006; Corre et al., 2009; Prakash et al., 2009), six for 16T allele (Harris et al., 2005; Sundblad et al., 2006; Corre et al., 2009; Woad et al., 2009) and five for 124G allele (Dixit et al., 2006; Corre et al., 2009; Woad et al., 2009). Dixit et al. (2006) did not specify the number of mutant type homozygous (16T/T) and therefore it was omitted from the analysis of C16T polymorphism.

The studies were conducted in various populations with different ethnic groups: six involved Europeans (Shelling et al., 2000; Harris et al., 2005; Corre et al., 2009; Woad et al., 2009), one East Asians (Jeong et al., 2004), two Asian Indians (Dixit et al., 2006; Prakash et al., 2009), and in one study the ethnicity was unspecified with study area being the Argentina (Sundblad et al., 2006). None of the studies stated that controls were aged matched. In all studies, it was reported that standard genotyping methods were used.

Summary statistics

Overall, the studies provided 1030/1660 cases/controls for G769A polymorphism, 936/1398 cases/controls for C16T polymorphism and 938/1446 cases/controls for A124G polymorphism. The prevalence of carriers of the G769A variant was similar in cases with POF (5.0%) and controls (4.9%). The frequency of 16T allele was greater...
in cases (35%) than in controls (30%) and the frequency of 124G allele was similar in cases (41%) and controls (42%).

HWE was tested for eight studies (Harris et al., 2005; Dixit et al., 2006; Sundblad et al., 2006; Corre et al., 2009; Woad et al., 2009) since only those provided the complete genotype distribution, and in all of them the controls did not depart from HWE. The quality of the remaining studies cannot be examined and the meta-analysis results should be interpreted with caution.

Main and subgroup analyses

Figure 1 shows the results for the association between the different gene polymorphisms and the risk of POF. The overall analysis of the G769A polymorphism revealed significant heterogeneity among studies ($P_Q < 0.01$, $I^2 = 73\%$) and that carriership of the G769A variant did not increase the risk of POF: $OR = 1.38 (0.48–3.94)$. In subgroup analysis by ethnicity, there was non-significant heterogeneity between the studies performed in Europeans ($P_Q = 0.01$, $I^2 = 74\%$) and in Asian Indians ($P_Q = 0.14$). The association was non-significant in Europeans ($OR = 0.71 (0.23–2.21)$ whereas it was significant in Asian Indians ($OR = 8.10 (1.27–51.6)$), however, the later was based on two studies and results should be interpreted with caution.

Regarding the C16T polymorphism, the 16T allele was not associated with POF ($OR = 0.94 (0.76–1.16)$) and the heterogeneity among studies was not significant ($P_Q = 0.12$, $I^2 = 43\%$). In subgroup analysis, Europeans shown large heterogeneity ($P_Q = 0.07$, $I^2 = 55\%$) and non-significant association ($OR = 0.93 (0.72–1.18)$).

Finally, the overall analysis for the A124G polymorphism indicated that the 124G allele is not a significant risk factor for POF ($OR = 0.98 (0.86–1.11)$) and that there is lack of heterogeneity among studies ($P_Q = 0.37$, $I^2 = 7\%$). Europeans produced similar results to the overall analysis ($OR = 0.95 (0.84–1.08)$, $P_Q = 0.47$, $I^2 = 0\%$).

Potential bias

Whether genotyping was blinded to clinical status was not disclosed in any of the studies. Cumulative meta-analysis for G769A polymorphism showed a downward trend of the genetic risk which was not significant in the whole studied period (Fig. 2). For C16T polymorphism, the genetic risk had a (upward) trend towards to non-significance and only in the first year it was significant (Fig. 2).

The recursive cumulative meta-analysis for G769A and C16T polymorphisms showed that the risk effect did not stabilize in a specific value (for carriers of the G769A variant the changes in OR at 2009/2006, 2006/2004 and 2004/2000 were 0.29, 0.74 and 0.57, respectively, and for 16T allele the changes at 2009/2006 and 2006/2005 were 1.36 and 1.44, respectively) indicating that more evidence is needed to draw safer conclusions regarding the effect sizes.

The Egger test for INHA G769A and C16T polymorphisms showed that there was no differential magnitude of effect in large versus small studies ($P = 0.26$ and $P = 0.17$, respectively).

Discussion

The present meta-analysis examined the INHA G769A, C16T and A124G gene polymorphisms and their relationship to risk of POF. The strength of the present analysis was based on the accumulation of published data giving greater information to detect significant differences. Synthesis of data from many studies is expected to improve power and reduce false discovery rate in all circumstances and the
gain could be considerable when there is lack of heterogeneity (Hedges and Pigott, 2001). However, power calculations are considered inappropriate in meta-analysis since that data are already assembled (Zintzaras and Lau, 2008a).

In the present study the genetic risk effect of carriehership of the G769A variant and the effect of 16T and 124G alleles were estimated. The meta-analysis showed that the investigated polymorphisms were not associated with POF. Only for G769A in Asian Indians an association was indicated, however, the result was based on very few studies and subjects. Overall, the pooled OR excluded with 95% certainty that carriers of the G769A variant would have more than 186% increased odds for POF or less than 65%. For C16T and A124G polymorphisms, the pooled OR excluded with 95% certainty that 16T allele would have more than 23% increased odds for POF or less than 18% and that 124G allele would have more than 137% increased odds for POF or less than 12%.

The meta-analysis results were based on a relatively small number of studies and they should be interpreted with caution. Although, there was lack of heterogeneity, the independent studies produced different results and hypothesis-generating findings were not replicated across several studies. Furthermore, the population diversity influenced the effect sizes of G769A polymorphism and there was inconsistency of genetic effects across ethnicities (Europeans and Asian Indians). For the other two polymorphisms, the consistency of the genetic effects across the different population groups does not necessarily mean that ‘race’ specific genetic effects are exactly the same since the number of studies and participants in each population was limited (Zintzaras and Lau, 2008a). There is no potential source of bias since there was no differential magnitude of effect in large versus small studies. The cumulative and recursive cumulative meta-analysis indicated that more evidence is required to draw definite claims for association since the risk effects did not reach stability.

The overall lack of association between the polymorphisms and POF might be due to other unidentified functional mutations that exist in the INHA gene that affect the susceptibility to POF. In addition, other genes (such as FSHR and FOXL2) involved in the FSH pathway may affect the risk of POF (Simpson, 2008; Baysen et al., 2009; Laissue et al., 2009). Discrepant results could be due to other loci that may have an effect on the genetic susceptibility to POF that are also in linkage disequilibrium with the examined polymorphisms of INHA gene (Shelling et al., 2000). In particular, the 769A variant is quite rare in all populations and all the studies included in the meta-analysis involved a small number of subjects and therefore, the analyzed sample may not be very representative of the different populations considered. Thus, the frequency of the variant will have to be determined in a larger number of samples.

The interaction of gene polymorphisms can be a major determinant of POF risk and individual polymorphisms might not be reliable markers of POF (Zintzaras and Lau, 2008a). If so, a meta-analysis of genotype combinations, or haplotypes, may provide more reliable information than single gene polymorphisms (Zintzaras et al., 2006). The search for susceptibility loci in POF has probably been complicated by the increased number of contributing loci and susceptibility alleles (Dixit et al., 2006). Furthermore, the published studies reported different set of SNPs making direct comparisons across studies difficult in the context of the meta-analysis. However, elucidating the pathogenesis of POF would demand investigation of association for many genetic variants of genes which constitute distinct pathophysiological pathways in the context of field synopsis and synthesis (Zdoukopoulos and Zintzaras, 2008).

Elucidating the genetics of POF largely relies upon the designing and undertaking of rigorous candidate-gene association studies. In future, studies should be planned with the idea of being incorporated with other similar studies in a meta-analysis. Meta-analysis also offers the opportunity to place each study in the context of all others and to examine why studies reach different conclusions (Zintzaras and Lau, 2008b). In addition to candidate gene approaches, genomic expression analyses and genome-wide association studies may assist in the selection of candidate variants by identifying genes involved in POF pathogenesis. Then, a possible ‘genomic convergence’ may provide further insights into the molecular mechanisms involved in POF (Kitsios and Zintzaras, 2009).

In summary, the accumulated evidence indicated lack of association for INHA G769A, C16T and A124G polymorphisms. However, the results of the present meta-analysis were based on relatively small numbers of studies and subjects, and consequently, their interpretation has to be cautious. Taking into account that POF is a complex disease with multifactorial etiology, the contributing pathogenic role of all the investigated gene polymorphisms cannot be excluded. Large long-term prospective and case–control studies that investigate combinations of gene polymorphisms (Cordell and Clayton, 2005) and gene-environment interaction (Clayton and McKeigue, 2001) will help to elucidate the genetics of POF further.
References


Submitted on April 12, 2009; resubmitted on June 12, 2009; accepted on June 16, 2009