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Polymorphisms of the insulin receptor and the insulin receptor substrates genes in polycystic ovary syndrome: A Mendelian randomization meta-analysis

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a heterogeneous condition with unknown aetiology which is considered to be the most common endocrine disorder in women of reproductive age. In this work we investigated the association of insulin receptor (INSR) and insulin receptor substrates (IRSs) polymorphisms with the risk of developing PCOS. The meta-analysis of eleven studies (889 cases, 1303 controls) yielded a significant association for IRS-1 Gly972Arg (G972R) polymorphism concerning the GR vs. GG genotype (OR: 1.77, 95% CI: 1.28, 2.45), with no between-studies heterogeneity. Concerning INSR His1058 C/T, the meta-analysis of eight studies (795 cases, 576 controls) found no significant evidence for association with PCOS (OR for the TT + CT vs. CC comparison equal to 1.28 with 95% CI: 0.88, 1.85) and a moderate between studies variability (I^2 = 44.6%). No evidence for publication bias was found in these meta-analyses. Following a multivariate Mendelian randomization approach, the overall OR was unaffected but the overall mean difference of fasting insulin levels between carriers of GR and RR genotypes in controls was significant (2.18, 95% CI: 0.36, 4.01). These results suggest that IRS-1 Gly972Arg polymorphism is significantly associated with the risk of developing PCOS and that this association is primarily mediated by increasing the levels of fasting insulin. The particular polymorphism is located in a region nearby two phosphorylation sites that interact physically with INSR and PI 3-kinase and there is enough evidence from the literature suggesting that the Arg972 variant is associated with decreased PI 3-kinase activity and impaired insulin-stimulated signaling.

Introduction

Polycystic ovary syndrome (PCOS) is considered to be the most common endocrine disorder in women of reproductive age [1–3]. PCOS is a heterogeneous condition characterized by chronic anovulation, irregular menses, infertility, hyperandrogenism and insulin resistance [1,4]. The prevalence of PCOS is estimated to be approximately ~7% in the general population in populations of Caucasian [2,5,6] or African–american origin [7], although in some Asian populations the prevalence is reported to be significantly lower [8]. The fundamental defect that causes PCOS remains elusive and it is believed to be multifactorial in origin, where environmental factors are acting in a genetic background, resulting in a broad spectrum of reproductive, as well as metabolic, defects [1,9]. Although infertility is the most common disorder associated with the syndrome [10,11], women with PCOS are also more likely to develop components of the metabolic syndrome such as diabetes, obesity, hypertension and dyslipidemia [12,13], which in turn are major risk factors for cardiovascular disease [14].

Various genetic markers have been implicated in the predisposition to PCOS; however, no single variant has conclusively and repeatedly been associated with the syndrome [15–17]. Insulin receptor (INSR) has been detected in the ovarian stroma, a finding consistent with the hypothesis that insulin can directly influence ovarian function by stimulating steroidogenesis [18]. In vitro and in vivo experiments indicate that insulin enhances ovarian growth and cyst formation [19]. The insulin receptor gene is composed of 22 exons spanning over 120 kb on chromosome 19 [20]. Mutations on exons 17–21, which encode the tyrosine kinase domain of the receptor, are associated with moderate hyperinsulinaemia and insulin resistance expressed in the heterozygous state [21,22]. Several kinds of polymorphisms have been identified within the coding and non-coding regions of INSR in patients with PCOS [23–25] most of which were silent single nucleotide polymorphisms (SNPs) [24,26]. A number of linkage and association studies have been carried out by different researchers attempting to associate various SNPs with PCOS [27]. One of them was the silent C10923T single nucleotide polymorphism at exon 17 [24,25,28–31], where we have a C-to-T substitution at His1058, which is located in the tyrosine kinase domain of insulin receptor and it is critical to the function of the gene. His1058 C/T polymorphism impinges on the

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operation of tyrosine kinase autophosphorylation of the insulin receptor in women with PCOS [32].

Insulin receptor substrate (IRS) molecules are key mediators in insulin signaling and act as docking proteins between the insulin receptor and a complex network of intracellular signaling molecules containing Src homology 2 (SH2) domains [33]. Four members (IRS-1, IRS-2, IRS-3, IRS-4) of this family have been identified and various polymorphisms have been determined in IRS-1 and IRS-2 [34]. IRS-1 is the major cytosolic substrate of the insulin receptor and is required for several effects of insulin such as glucose transport [35]. When IRS-1 is dysfunctional, IRS-2 is the main docking protein for the intracellular propagation of the insulin signal [35]. The Gly972Arg (G972R) polymorphism [36–46] is the more frequent polymorphism of IRS-1 and has been associated with PCOS and type 2 diabetes [47,48].

Since the first publications concerning INSR His1058 C/T [28] and IRS-1 Gly972Arg [36] polymorphisms with PCOS, several attempts have been made in order to elucidate these findings, but the results were controversial. In this work, a comprehensive literature search and a meta-analysis was performed for investigating the association of INSR and IRSs polymorphisms with the development of PCOS.

Material and methods

Retrieval of published studies

A comprehensive electronic search of PubMed and Scopus was conducted up until May 2009. The following keywords or combination of them were used: "insulin receptor", "INSR", "insulin receptor substrate", "IRS", "polymorphism", "allele" etc. coupled with the term "polycystic ovary syndrome" or "polycystic ovaries". The electronic investigation was supplemented by the assessment of the references of published studies and the manual search of abstracts from conference meetings.

Studies were included in the analysis if: (i) they examined the contribution of the either INSR or IRSs polymorphisms to the development of PCOS and (ii) they provided adequate data to calculate an estimate of relative risk (RR) comparing PCOS patients and healthy unrelated controls. Family-based studies that use the Transmission Disequilibrium Test (TDT) were not included. To avoid selection bias, published manuscripts were considered for review without any language or quality restrictions [49,50]. Furthermore, to eliminate "grey literature" bias [51], we decided to include studies published in conference proceedings or as short abstracts (if found). Extraction of summary measures for phenotypic characteristics such as fasting insulin levels, fasting glucose levels, body mass index (BMI), dehydroepiandrosterone sulfate (DHEAS) and testosterone levels was also performed separately for cases and controls in order to perform meta-regression analysis and to integrate them in the Mendelian randomization approach (see below). When data were reported on different measurement units, standard transformation was performed and the respective variances were calculated.

Statistical analysis

Odds ratio (OR) was the metric of choice in all contrasts assessed. In case of zero cell counts a continuity correction was applied by adding 0.5 to all cells of the contingency table. The between-study heterogeneity was evaluated using the chi-square based Cochran's Q statistic [52] and the inconsistency index (I^2) [53]. Data were combined using random-effects models with inverse-variance weights. For implementing the random-effects method and for estimating the between-studies variance (τ^2), we used the widely applicable non-iterative method of moments proposed by DerSimonian and Laird [54]. The method is very simple and uses the Cochran's Q statistic in order to calculate an estimate of τ^2. In case of heterogeneity, random-effects models are more appropriate since they usually are more conservative, whereas when heterogeneity is absent (i.e. when Q and I^2 are equal to zero), random- and fixed-effects methods coincide. More advanced methods for multivariate meta-analysis of gene-disease association studies that use iterative schemes, avoid the multiple comparisons and test directly the genetic model were also implemented [55–57]. Deviations from Hardy–Weinberg equilibrium (HWE) were calculated by the chi-square method, and sensitivity analyses were performed for assessing the impact of studies that deviate significantly [58].

For the Mendelian randomization approach we used the multivariate method proposed by Minelli et al. [59,60]. The Mendelian randomization aims at determining the causal role of an intermediate phenotype in the risk of developing the disease. Thus, with this approach apart from being able to decipher whether a polymorphism predisposes to a disease, we can also infer the causal pathway. In brief, we modeled simultaneously in a multivariate framework the genotype-disease and genotype–phenotype association. For genotype-disease association we used the logOR for a contrast of a mutant genotype as described in the previous paragraph. As a carefully selected phenotype we decided to use the fasting insulin levels in controls, since the Gly972Arg polymorphism is located in a region nearby two phosphorylation sites that interact physically with INSR and PI 3-kinase [61] and there is enough evidence from the literature suggesting that the Arg972 variant is associated with decreased PI 3-kinase activity and impaired insulin-stimulated signaling [62].

With the Mendelian randomization approach, in principle and under certain assumptions [63], we could decipher the causal pathway leading from the genotype to the formation of the syndrome and in particular, to investigate whether development of PCOS is directly promoted by increased insulin resistance and thus, whether it is linked with the development of diabetes. We used the model B as described in [60], since this model makes the assumption that heterogeneity in the measure of association of phenotype with genotype is independent of the heterogeneity in the logOR of phenotype on disease (i.e. the between-studies correlation equals to zero). Model A which is a simple bivariate model assuming only no within-studies correlation, yield results that depend on the between-studies correlation and there is unlikely to have sufficient information to estimate it accurately [60].

Publication bias or other small-studies related bias was assessed by the Begg's rank correlation method [64], the Egger's fixed-effects regression method [65] and its random-effects analogue [66]. In an attempt to identify potential influential studies, we calculated the effects estimates by removing an individual study each time and then checked if the overall significance of the estimate or of the heterogeneity statistic was altered. Cumulative meta-analysis [67,68] was performed in order to identify a possible trend of the overall OR over years [69]. For all analyses performed here, Stata 10 (StataCorp) was used. Statistically significant results were regarded those with a p-value <0.05.

Results

After the initial search we came up with 34 published studies that investigated the association of polymorphisms of insulin receptor and the respective substrates with PCOS. The majority of the polymorphisms that were identified were polymorphisms of INSR and IRS-1, although few polymorphisms of IRS-2 (i.e. Gly818Arg, Leu647Val, Gly879Ser [37]) were found also. The