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Germline Mutations of Inhibins in Early-Onset Ovarian Epithelial Tumors

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ABSTRACT: To identify novel genetic bases of early-onset epithelial ovarian tumors, we used the trio exome sequencing strategy in a patient without familial history of cancer who presented metastatic serous ovarian adenocarcinomas at 21 years of age. We identified a single de novo mutation (c.179G>T/p.Arg60Leu) within the INHA gene encoding the βA-subunit of inhibins/activins, which play a key role in ovarian development. In vitro, this mutation alters the ratio of secreted activins and inhibins. In a second patient with early-onset serous borderline papillary cystadenoma, we identified an unreported germline mutation (c.1157A>G/p.Asn386Ser) within the INHBA gene encoding the α-subunit, the partner of the βA-subunit. This mutation also alters the secreted activin/inhibin ratio, by disrupting both inhibin A and inhibin B biosynthesis. In a cohort of 62 cases, we detected an unreported germline mutation of the INHA gene (c.839G>A/p.Gly280Glu). Our results strongly suggest that inhibin mutations contribute to the genetic determinism of epithelial ovarian tumors.

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KEY WORDS: cancer; ovary; exome; inhibin; activin; INHA; INHBA

In many families suspected to present an inherited form of cancer, analyses focused on genes known to be involved in Mendelian predisposition to cancer often remain negative, despite a personal and/or familial history strongly suggestive of a genetic determinism. Therefore, other pathways involved in cancer genetics remain to be identified. We considered that the comparative index-case-parents exome sequencing strategy, originally developed to detect de novo mutation in early-onset severe conditions such as mental retardation [Vissers et al., 2010], could be a powerful method to identify novel molecular bases of cancer. Indeed, given the numerous examples of de novo mutations involved in genetic conditions recently described in humans [Veltman and Brunner, 2012], a fraction of early-onset sporadic cancer cases could result from de novo germline mutations affecting unknown cancer predisposing genes. The genes affected by these de novo mutations may represent novel candidate genes for familial forms of cancer.

For this study, we used as a paradigm sporadic early-onset epithelial ovarian cancer. In the general population, epithelial ovarian tumors usually occur after 50 years of age and the development of epithelial ovarian tumors before the age of 40 is therefore suggestive, in clinical practice, of a genetic determinism. Early-onset epithelial ovarian tumors occur in patients with germline mutations of BRCA1, BRCA2, and the MMR genes [Lynch et al., 2009], and recently, germline mutations of other genes known to be involved in cancer such as BARD1, BRI1P1, CHEK2, MRE11A, NBN, PALB2, RAD50, RAD51C, RAD51D, or TP53 have been reported in patients with early-onset epithelial ovarian cancers [Loveday et al., 2011; Walsh et al., 2011]. Nevertheless, in a fraction of patients developing early-onset epithelial ovarian tumors, genetic analyses of these genes remain negative, suggesting the existence of uncharacterized deleterious mutations in other genes. We report here the identification by exome sequencing of a de novo germline mutation affecting the βA-subunit of inhibins/activins in a sporadic case of early-onset serous ovarian cancer and provide arguments indicating that germline inhibin mutations contribute to the genetic determinism of epithelial ovarian tumors by altering the inhibin/activin production.

The patient is a French woman of Caucasian origin who had developed at 21 years of age large bilateral ovarian tumors corresponding to serous low-grade ovarian adenocarcinomas (FIGO IV). These tumors were complicated by numerous peritoneal, uterine, colorectal, and pancreatic metastases that lead to a radical surgery followed by chemotherapy. Her father and mother, at 56 and 52 years of age, respectively, had neither personal nor familial history of cancer.

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cancer. In cancer genetics, the development of a bilateral ovarian adenocarcinoma at 21 years of age is strongly suggestive of a genetic predisposition. Screening for BRCA1, BRCA2 and TP53 point mutations and genomic rearrangements in the index case peripheral blood DNA had revealed no germline mutation. Considering the absence of cancer in the family, we speculated that a germline de novo mutation could be responsible for the early-onset of the tumors observed in this patient. To identify such mutations, we performed a comparative exome analysis of the patient and her healthy parents (Supp. Materials and Methods). For the parents, exome sequencing was performed on peripheral blood DNA. For the index case, the exome was performed on genomic DNA extracted from nontumoral ovarian tissue to be able to detect also postzygotic de novo mutations that may be present only in the ovarian tissue and could be missed by analyzing the peripheral blood DNA. Across the three exomes, we obtained an average of 8 Gb with 98% of mappable sequences, a mean Read Depth of 69x, 88% of bases were covered to a minimum depth of 10x and 89% of the read bases had a Qscore above 30. On average, 17,395 exonic variations were identified per exome. First, variants with a read coverage of less than 10x and a Qscore <30 were filtered out. We first checked in the index case exome the absence of deleterious mutations within the different genes known to be involved in the genetic determinism of ovarian cancer [Lynch et al., 2009; Loveday et al., 2011; Walsh et al., 2011].

Then, using the Exome Variation Analyser software [Coutant et al., 2012], we filtered the variations against the 1000 Genomes Project data set (May 2011, 20101123 release) and the ESP cohort data set (ESP6500), using an allelic frequency filter of 0.001. Variations present in our in-house database including 72 exomes were also filtered out. With these additional filters, an average of 295 novel variations was retained per individual. When we subtracted the variants detected in the parents from those detected in the index case to detect de novo mutations, 46 variations remained and were analyzed in more detail. Visualization of the BAM files revealed that 37/46 variations were in fact present in a low fraction of the reads obtained in the parents. Among the remaining variations, eight were detected in less than 20% of the reads and Sanger sequencing performed on such variations did not confirm their existence. These variations likely correspond to sequencing artifacts. After this filtering scheme, only one de novo mutation was detected in the patient exome: the c.1157A–G/p.Asn386Ser mutation, within the INHBA gene (NM_002192.2; MIM #147290; www.lovd.nl/inhba) encoding the βA-subunit of inhibin/activin proteins that play a key role in ovarian development and the functional impact of this mutation are two key arguments indicating that INHBA mutation, identified as the single de novo mutation in a sporadic bilateral epithelial ovarian cancer occurring at 21 years of age, is not related to ovarian cancer and that more relevant mutations were missed in the 12% fraction analyzed at lower coverage in the trio exomes.

To determine the impact of the detected INHBA mutation, we performed functional assays in HEK-293F cells (Supp. Materials and Methods). After transient cotransfection of cells with plasmids expressing wild-type α-subunit and wild-type or mutant (p.Asn386Ser) βA-subunits, we determined the quantitative effect of the mutation on the activin A and inhibin A production in conditioned media, using an E4/E4 and an E4/R1 immunoassay, respectively, as previously described [Makanji et al., 2008]. As shown in Figure 1, we observed for the p.Asn386Ser mutant as compared with the wild-type βA-subunit, a 28% decrease in activin A production and a 44% increase in inhibin A production (P = 0.0007 and P = 0.048, respectively, two-tailed t-test). Activin A has been shown to inhibit the growth of epithelial cells including breast cancer cells and ovarian cancer cells through Smads and CDK inhibitor activation [Burdette et al., 2005; Ramachandran et al., 2009] suggesting therefore that a reduction of activin A, such as the one observed with the INHBA p.Asn386Ser mutation, could favor the growth of epithelial ovarian cells. Therefore, the identification in an early-onset sporadic bilateral ovarian adenocarcinoma case of a single de novo mutation of a gene playing a pivotal role in ovarian development and the functional impact of this mutation are two key arguments indicating that germline INHBA mutation can cause, alone or in combination with other transmitted genetic variations, early-onset epithelial ovarian cancer.

To determine the prevalence of germline INHBA mutations in early-onset ovarian tumors, we then screened for germline INHBA mutations by Sanger sequencing 43 other patients (14 originated from France and 29 from USA), who had developed ovarian epithelial tumors before 40 years of age and in whom analysis of BRCA1 and BRCA2 genes had revealed no deleterious mutation. We observed no additional mutation of the INHBA gene in these 43 patients. Since the identified de novo mutation of INHBA...
The impact of the INHA p.Arg60Leu mutation was confirmed in the ovarian tumor between the total replication cohort of 76 patients. We detected within the INHA and INHBA genes are related to rare variants. The Gly280 residue lies within the prodomain of the α-subunit that encodes the partner of INHA and INHBA, which encode the subunit and the wild-type βA- or βB-subunits, were measured using an E4/R1 immunoassay and a C5/R1 immunoassay, respectively. The results correspond to six independent transfections.\(^*\), Two-tailed t-test at P < 0.05.

Figure 2. The impact of the INHA p.Arg60Leu mutation on the amount of secreted inhibins A and B. Activin A and B concentrations in the conditioned media of HEK-293F cells, 2 days after cotransfection of plasmids expressing the wild-type or mutant (p.Arg60Leu) inhibin α-subunit and the wild-type βA- or βB-subunits, were measured using an E4/R1 immunoassay and a C5/R1 immunoassay, respectively. The results correspond to six independent transfections.\(^*\), Two-tailed t-test at P < 0.05.

 alters the ratio of inhibin A versus activin A and the βA-subunit heterodimerizes with the α-subunit to form inhibin A, we also screened the 14 French patients for germline mutations of the INHA gene encoding the α-subunit. This analysis revealed, in a Caucasian patient who presented at 29 years of age a serous borderline papillary cystadenoma, a germline heterozygous mutation c.179G>T/p.Arg60Leu (NM_000219.1; www.lovd.nl/INHA), which had not been previously reported in the different databases. DNA from the unaffected parents was not available. The presence of this heterozygous INHA mutation was confirmed in the ovarian tumor of the carrier. Arg60 is a very conserved residue that forms part of a secondary furin cleavage site located at the amino terminus of the inhibin α-prodomain [Walton et al., 2012]. The measurement, using immunoassays, of inhibin A and B production in conditioned media from HEK-293F cells cotransfected with plasmids expressing wild-type or mutant α-subunit and wild-type βA- or βB-subunit (Fig. 2), revealed that the p.Arg60Leu INHA mutation disrupts both inhibin A biosynthesis (75% decrease, P = 0.00006) and inhibin B biosynthesis (55% decrease, P = 0.002). Remarkably, INHA→female mice specifically develop mixed ovarian tumors indicating that INHA could act as a tumor suppressor gene with gonadal specificity [Matzuk et al., 1992], which is in agreement with the possible oncogenic effect of the p.Arg60Leu INHA gene detected in this study.

Exome sequencing data are available for a cohort of 316 high-grade serous ovarian tumors [The Cancer Genome Atlas Network, 2011]. Somatic alterations of the INHA or INHBA genes are reported in 5% of the cases but no germline mutation of these genes is reported. Because this TCGA cohort is not enriched in early-onset cases but rather reflects the overall age distribution of serous ovarian cancer, we decided to complement the INHBA and INHA mutation screening by Sanger sequencing a second series of 62 other French early-onset ovarian epithelial cases (40 years), without detectable BRCA mutation. We detected within the INHBA gene an additional new germline heterozygous variation, c.839G>A, p.Gly280Glu (NM_000219.2). This variation was detected in a patient who developed a serous papillary ovarian adenocarcinoma at 27 years of age. DNA sample from the unaffected parents was not available.

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