No evidence of somatic DNA copy number alterations in eutopic and ectopic endometrial tissue in endometriosis

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BACKGROUND: *De novo* somatic copy number aberrations (SCNAs) in eutopic and ectopic endometria are thought to be involved in the pathogenesis of endometriosis. In this study we used, for the first time, high-density single nucleotide polymorphism-array technology for accurate detection of SCNAs, inherited DNA copy number variations (CNVs) and copy-neutral loss of heterozygosity (cn-LOH) patterns in patients with endometriosis.

METHODS: The Illumina HumanOmniExpress array was used to detect *de novo* somatic genomic alterations in eutopic and ectopic endometria from 11 women (eight with Stage I–II endometriosis and three with Stage III–IV endometriosis) by comparatively analysing DNA from peripheral blood, eutopic endometrium and a pure population of endometriotic cells harvested from endometriotic lesions by laser capture microdissection (LCM). The frequency of the CNV in 3p14.1 from blood DNA of 187 endometriosis patients (94 with Stage I–II endometriosis and 93 with Stage III–IV endometriosis) and 171 healthy women from the Estonian general population was evaluated.

RESULTS: Analysis of array data showed that LCM DNA can be used successfully for detection of genetic changes as all inherited CNVs were identified in all tissues studied. No unique SCNAs or cases of cn-LOH were found in either eutopic or ectopic endometrium when compared with blood DNA. The frequency of the deletion allele in 3p14.1 did not differ between studied groups.

CONCLUSIONS: In the present study no endometriosis-specific SCNAs or regions of cn-LOH in eutopic or ectopic endometrium were found. Nevertheless, as we studied only 17 endometriotic tissues derived from 11 patients we cannot entirely exclude the occurrence of rare SCNAs. Based on our results we suggest that molecular mechanisms other than chromosomal rearrangements most likely underlie the onset and progression of endometriosis.

Key words: endometriosis / copy number variations / somatic copy number aberration / single nucleotide polymorphism / ectopic endometrium

Introduction

The concept that endometriosis is a tumor-like disease because of its metastatic potential, local tissue invasion and increased growth and vascularization of ectopic endometrial tissue is widespread and generally accepted. Numerous studies have relied on the idea that endometriosis has similarities with malignant diseases, and have found evidence that unique tissue-specific *de novo* somatic DNA copy number alterations (SCNAs), characteristic of various neoplasms, are also present in endometriotic lesions (Sato *et al.*, 2000; Goumenou *et al.*, 2001; Guo *et al.*, 2004; Wu *et al.*, 2006, Veiga-Castelli *et al.*, 2010). Alternatively, inherited chromosomal DNA copy number variations (CNVs) that exist in all normal tissues of the body have been in some cases associated with the

genetic susceptibility to cancer development (reviewed in Kuiper et al., 2010). Besides SCNAs and CNVs, acquired copy-neutral loss of heterozygosity (cn-LOH) events in somatic tissues, characterized by deletion of one copy and compensatory duplication of the other allele, are also associated with the pathogenesis of a wide range of cancers (reviewed in Makishima and Maciejewski, 2011). However, the cn-LOH profile in endometriosis patients has not been described previously and the relevance of extended homozygosity regions in the pathogenesis of endometriosis is unknown.

Previous genome-wide studies focusing on genomic alterations in endometriotic foci or endometria of endometriosis patients, or studies comparing changes in eutopic and ectopic endometria, have shown various chromosomal alterations but only a few of these alterations were observed in more than one study (Table I; Gogusev et al., 1999, Guo et al., 2004; Wu et al., 2006; Veiga-Castelli et al., 2010). Furthermore, not all investigators identified chromosomal aberrations in ectopic endometrial tissue or eutopic endometrium of endometriosis patients (Prowse et al., 2005; Zafrakas et al., 2008), thus raising a question about the relevance of DNA genomic imbalance in the pathogenesis of endometriosis.

In studies of endometriosis, little attention has been paid to the histological examination of biopsy samples. The importance of histological examination is illustrated by the fact that in many biopsy samples with a 'typical' macroscopic appearance of an endometriotic lesion, the diagnosis cannot be confirmed at a microscopic level (Moen and Halvorsen, 1992; Walter et al., 2001). This could be because of the very small size of the lesions or because samples taken may lack endometriotic glands, and sparse stromal cells may be hidden in surrounding tissue (Kennedy et al., 2005). Therefore, it is highly probable that genetic alterations can be missed because of an insufficient amount of endometriosis-specific cells in standard biopsy tissue samples. Laser capture microdissection (LCM) is a powerful tool for isolating specific cells of interest from microscopic regions of tissue, and thus enables the study of endometriosis-specific cells without the confounding surrounding tissue.

Earlier studies have used different methods (e.g. karyotype analysis, comparative genomic hybridization (CGH), fluorescence in-situ hybridization and microsatellite analysis) to detect tissue-specific genomic alterations in eutopic or ectopic tissue from patients with endometriosis. However, to the best of our knowledge, none of these studies have compared the genomic alterations of eutopic or ectopic endometrium with those in DNA extracted from the blood of the same individual. Therefore, it is possible that many of the described genomic alterations reported previously in eutopic or ectopic endometrium actually represent CNVs that are present in all tissues of studied individuals, rather than endometriosis lesion-specific SCNAs. Owing to major improvements in molecular genetic techniques, high-resolution single nucleotide polymorphism (SNP) arrays provide opportunities for more precise identification of chromosomal aberrations and cn-LOH regions. Therefore, in this study we combined for the first time LCM and SNP-array analysis to study de novo SCNAs, CNVs and cn-LOH patterns in eutopic or ectopic endometrium and blood cells in order to test the hypothesis that DNA variability in endometriotic lesions and eutopic endometrium is critical for endometriosis development.

Materials and Methods

Patients and tissue samples

Eleven patients with a diagnosis of endometriosis undergoing laparoscopy at the Tartu University Hospital Women's Clinic and Elite Clinic (Tartu, Estonia) were recruited into the study. None of the patients had received any hormonal treatment for at least 3 months prior to the laparoscopy. The severity of endometriosis was classified according to the American Society for Reproductive Medicine revised classification system (ASRM,

Table I Overview of alterations in genome-wide copy number in studies of endometriosis in women.

Type of studied tissue	SCNA detection method	Controls	Patients with alterations (n)	Total SCNAs (n)	Frequent SCNAs − (loss); + (gain)	Reference
Ectopic (n = 10); eutopic (n = 10)	CGH		5/10	15 ectopic; 19 eutopic	+11q, +17p, +17q, +19p	Veiga-Castelli et al. (2010)
Ectopic (n = 10)	aCGH		0/10			Zafrakas et al. (2008)
Ectopic (n = 5); eutopic (n = 5)	aCGH	4 endometria from healthy women	5/5	810 ectopic; 745 eutopic	+1p, +6p, +6q, +11p, +Xq, −1p, −5p, −6q, −16q	Wu et al. (2006)
Eutopic (n = 5)	aCGH	4 endometria from healthy women and 1 placenta	5/5	68	+3p, +10q, +13q, −1p, −3p, −4p, −22q	Guo et al. (2004)
Ectopic (n = 18)	CGH		15/18	59	+1q, +6q, +7q, +17q, −1p, −5p, −6q, −7p, −22q, −9q, −16q, −17q	Gogusev et al. (1999)
Ectopic (17); eutopic (11); blood (11)	SNP array	Peripheral blood of the same patient	0/11			Our study

SCNA, somatic copy number aberration; SNP, single nucleotide polymorphism; aCGH, array comparative genomic hybridization.

1997; Table II). Peripheral blood samples were taken before surgery from all participating patients. Endometrial biopsy samples were obtained using an endometrial suction catheter (Pipelle, Laboratoire CCD, France), while endometriotic lesions were collected during laparoscopic surgery. Biopsies were snap-frozen immediately in liquid nitrogen and stored at −80°C until use. Histological sections (10 µm) of endometriotic tissue (embedded in OCT: Leica, Germany) were mounted on PEN membrane (P.A.L.M. Mikrolaser Technology, Germany) microscope slides and lightly stained with hematoxylin/eosin. LCM of endometriotic cells (glandular epithelial cells together with a small amount of surrounding stromal cells) from endometriotic foci was performed using a PALM laser (MicroBeam, P.A.L.M. Mikrolaser Technology; Fig. 1) according to the manufacturer's instructions. Genomic DNA from peripheral EDTA-blood, endometrial tissue and endometriotic cells harvested following LCM was isolated using a QIAamp DNA Mini kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions. DNA extracted from LCM samples was

Table II General characteristics of patients, biopsy samples analyzed and results obtained.

Patient ID	Age (years)	Endometriosis stage	Location of analyzed ectopic endometrial tissue	CNV (n)	cn-LOH regions with >5 Mb (n)
E1	37	III	Ligamentum sacrouterina	6	
			Peritoneum		
E2	41	III	Peritoneum	4	1
			Corpus uteri		
E3	30	II	Corpus uteri	4	
			Ligamentum sacrouterina		
E4	42	III	Ligamentum sacrouterina	11	
			Ovary		
E5	31	III	Ligamentum sacrouterina	6	
			Corpus uteri		
E6	38	III	Ligamentum sacrouterina	7	
E7	45	II	Ligamentum sacrouterina	1	1
			Ligamentum sacrouterina		
E8	35	III	Ovarian endometrioma	6	
E9	36	I	Ovary	5	
E10	41	IV	Corpus uteri		1
E11	29	IV	Ligamentum Sacrouterina	2	

CNVs and cn-LOHs were present in all tissue samples (blood, endometrium and endometriosis foci) from a patient.
CNV, copy number variations, Cn-LOH, copy-neutral loss of heterozygosity.
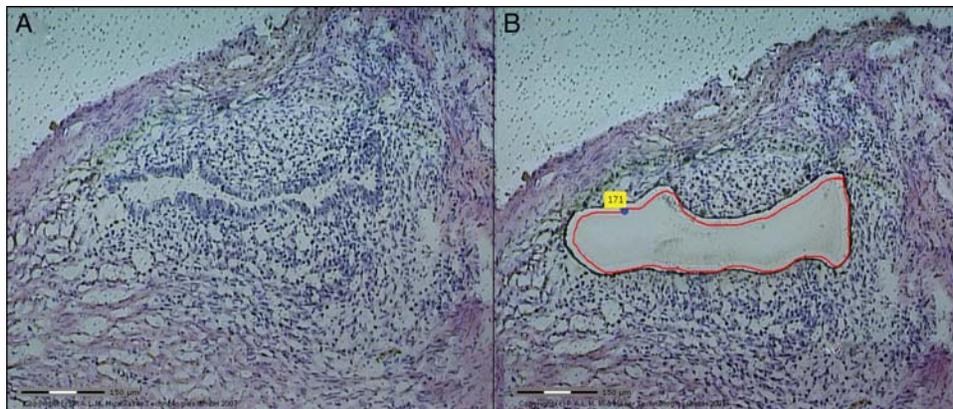

Figure 1 Representative photomicrographs of ectopic endometriosis foci (sections of *ligamentum sacrouterina*, 10× magnification, 10 µm hematoxylin and eosin-stained sections) before and after LCM. (**A**) Ectopic endometrial tissue before LCM, endometriotic gland surrounded by endometriotic stromal cells and peritoneal tissue. (**B**) Ectopic endometrial tissue after LCM.

concentrated using a rotational-vacuum-concentrator (RVC 2-25; Martin Christ Gefriertrocknungs, Germany). DNA concentrations were measured using a NanoDrop 2000 (Thermo Fisher Scientific, Inc. USA) spectrophotometer.

To investigate the frequency of the CNV in the 3p14.1 intergenic region downstream of potential tumor suppressor gene *MAGI1* (membrane-associated guanylate kinase, WW and PDZ-domain containing 1), the blood DNA of an additional 176 patients with endometriosis, among them 150 patients from our previous study (Saare et al., 2010), was analysed. Altogether 187 women (94 with Stage I–II endometriosis and 93 with Stage III–IV endometriosis, mean age: 32.6 ± 6.1 years) were studied. One hundred and seventy-one women (mean age: 36.0 ± 6.0 years) representing the Estonian general population and with no medical history of endometriosis were enrolled in the study as controls and their genomic DNA isolated from blood samples was obtained from the collection at the Estonian Genome Center of the University of Tartu (Tartu, Estonia).

The study was approved by the Ethics Review Committee on Human Research of the University of Tartu (Tartu, Estonia) and informed consent was obtained from all participants.

SNP arrays and data analysis

Eleven women with endometriosis were recruited for the SNP-array study. The HumanOmniExpress BeadChip (Illumina, Inc., San Diego, CA, USA) arrays with a total of 733 202 SNPs covering the whole genome at a median of 2.2 kb intervals were used for genotyping DNA from patients' blood ($n = 11$), endometrium ($n = 11$) and endometriotic foci ($n = 17$). Endometriotic foci samples (Table II) and matched blood and endometrial samples were run separately. The assay was performed according to the manufacturer's protocol and 200 ng of total DNA per sample was used.

All genotyped samples had a call rate >99% and were suitable for CNV analysis. First, CNV and cn-LOH regions were determined using GenomeStudio software, GT module v.3.1 (Illumina, Inc.). Second, to verify all CNVs found by GenomeStudio software and to possibly detect shorter scale CNVs, two independent DNA copy-number detection algorithms, PennCNV (Wang et al., 2007) and QuantiSNP (Colella et al., 2007), were used. For this, normalized signal intensities (log R ratios) and minor allele (B allele) frequencies for each marker were exported from GenomeStudio software. QuantiSNP was then utilized to identify cn-LOH regions for each tested sample. Genotyping data from the Estonian general population ($n = 1000$) were used as a reference (Nelis et al., 2009) in the PennCNV software. CNVs that were not detected by both PennCNV and QuantiSNP programs, which were <1 kb in size, and had a Log Bayes Factor <25 were excluded from further analysis. The length of cn-LOH regions was set at 5 Mb. To find causal CNVs, all identified CNVs were screened against known genomic variants in the Database of Genomic Variants (http://projects.tcag.ca/variation).

Quantitative real-time PCR

For each type of detected genomic rearrangement (hemizygous deletions, homozygous deletions and hemizygous duplications), the presence of some CNVs and putative SCNAs was validated using quantitative real-time PCR in a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, USA; list of used primers available upon request). Real-time PCR was carried out using 20 ng DNA, 250 nM forward and reverse primers, $1\times$ HOT FIREPol EvaGreen qPCR Mix Plus (Solis BioDyne, Tartu, Estonia) and water in a total reaction volume of 20 µl per well. For quantification, the target locus was compared with the locus outside the putative variation region (reference region) and relative copy numbers were normalized against normal control genomic DNA. Fold changes in DNA copy number were calculated using the $2^{-\Delta\Delta Ct}$

method (Moroni et al., 2005). To detect the 3p14.1 CNV, the following primers were used: forward primer 5′-CCAAGGTAACCATCCTGT TGA-3′ and reverse primer 5′-CACCCAGCTAGGGATTTGTG -3′, reference forward primer 5′-GTCAGTCTCATCTGCAAATA-3′ and reverse primer 5′-GTAATGAAGCATCTGATACC-3′.

Statistical analysis

The frequency of the 3p14.1 deletion CNV in endometriosis and control group was analyzed using the χ^2 test (PASW 18.0 software, SPSS, Inc., Chicago, IL, USA). A P value of < 0.05 was considered statistically significant.

Results

We examined genomic DNA in the blood ($n = 11$), endometrium ($n = 11$) and microdissected endometriotic foci ($n = 17$) from 11 patients with endometriosis, using HumanOmniExpress BeadChip SNP arrays. Patient characteristics, including age, stage of endometriosis and the locations of analyzed ectopic endometrial tissue, are presented in Table II.

Analysis of array data by both analysis programs (PennCNV and QuantiSNP) showed no lesion-specific SCNAs. However, in distinct ectopic lesions four different hemizygous deletions with borderline confidence thresholds were proposed by one or other of these data analysis programs but validation using quantitative real-time PCR did not confirm the presence of copy number changes in the ectopic tissue compared with blood DNA of the same individual (data not shown).

Altogether 52 (0–11 per sample) genomic CNVs with an average region size of 85 kb (ranging from 3 to 340 kb) were found in the matched blood, endometrial and ectopic foci samples (Table II; Fig. 2). Most of these CNVs were hemizygous microdeletions ($n = 22$) or hemizygous microduplications ($n = 20$), while 10 CNVs were homozygous deletions. Forty-eight of the 52 CNVs were described in the Database of Genomic Variants and therefore considered as common CNVs. Four CNVs were novel: two hemizygous microdeletions and two hemizygous duplications (Table III). Five CNVs (2p22.3, 3p14.1, 4q13.1, 5q15, 10q11.22) were present in more than one patient (Table IV). The frequency of the 3p14.1 region deletion was 45% (in 5 patients out of 11), which is considerably higher than the 8% reported in the Database of Genomic Variants. Therefore, we tested whether this constitutional CNV might be associated with genetic predisposition to endometriosis. Quantitative real-time PCR was used to analyze the frequency of the 3p14.1 deletion using blood DNA from an additional 176 endometriosis patients (187 altogether), and the results were compared with the data from 171 healthy control women from the Estonian general population. The frequency of the deletion allele in the patient group was similar to that in the control group (9.4 versus 6.4% respectively, $P \geq 0.05$). In addition, we saw no significant difference in the deletion frequency distribution when the patients were divided according to disease stage: I–II (94 patients) versus III–IV (93 patients) stage endometriosis.

SNP arrays revealed 3 cn-LOH regions present in all tissues studied (blood, eutopic and ectopic endometria) from 11 different individuals, and spanning >5 Mb: with 2 of them located in centromeric regions of chromosomes 3 (patient E7) and 10 (patient E2), and one in
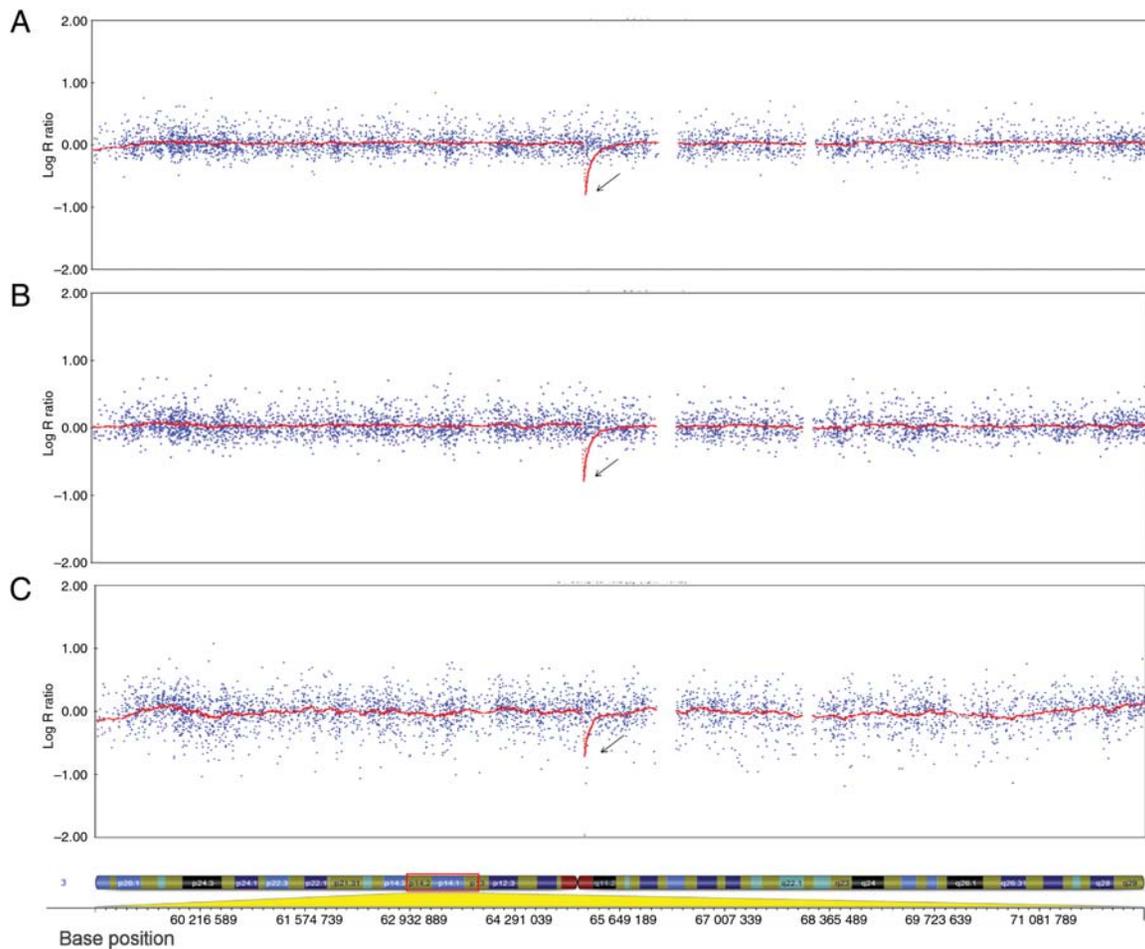
Figure 2 Visual microarray data analysis of patient E5: blood DNA (**A**), endometrial DNA (**B**) and LCM DNA of an endometriotic lesion (**C**). Copy number estimates (red line placed on log R ratio values) are shown for chromosome 3, positions 60 216 689–71 081 789 (image from BeadStudio Chromosome Browser). A homozygous deletion covering 22.8 kb in 3p14.1 is shown by arrows.

Table III Novel CNVs in women with endometriosis.

	Length (kb)	Genes	Patient ID
Hemizygous microduplication locus			
2p21	95.6	ABCG8	E6
14q12	41.8	BNIP 3P (pseudogene)	E8
Hemizygous microdeletion locus			
1q42.3	129.7	MTR	E4
10q23.1	14.0	NRG3	E6

CNVs were shared by all tissue samples (blood, endometrium and endometriosis foci) from a patient.

18q21.1-21.3 (patient E10; Table II). However, we detected no endometriotic foci-specific cn-LOH regions when DNA from lesions was compared with endometrial or blood DNA.

Discussion

To the best of our knowledge, this is the first study involving the use of LCM together with high-resolution SNP arrays to reveal somatic genomic alterations and cn-LOH in patients with endometriosis. The results of this study demonstrate that DNA from eutopic and ectopic endometria of patients with endometriosis showed no SCNAs or distinct cn-LOH patterns when compared with blood DNA. Therefore, we were unable to confirm the hypothesis that DNA variability in women with endometriosis is specific to endometriotic lesions and eutopic endometrium when compared with peripheral blood cells.

Currently, there is no clear consensus on the importance of SCNAs in endometriotic lesions or/and eutopic endometria in endometriosis patients (Table I). Gogusev et al. (1999) used CGH to analyze endometriotic lesions in 18 women with peritoneal, umbilical and ovarian endometriosis and reported loss/gain of genomic material at several chromosome regions. A later study by Guo et al. (2004) involved analysis of eutopic endometria from five patients with endometriosis with

Table IV CNVs shared between patients with endometriosis.

	Length (kb)	Genes	Patient ID
Homozygous microduplication locus			
10q11.21	160.5	*ANTXRL*	E4, E5, E7
Hemizygous microdeletion locus			
2p22.3	27.0	None	E9, E11
5q15	50.8	*LOC728048* (pseudogene)	E1, E4
Hemizygous or homozygous microdeletion locus			
4q13.1	12.3	None	E2[a], E4[b]
3p14.1	22.8	None	(E1, E2, E4)[a], (E5, E9)[b]

CNVs were shared by all tissue samples (blood, endometrium and endometriosis foci) from a patient.
[a]Hemizygous microdeletion.
[b]Homozygous microdeletion.

array CGH (aCGH) and various genomic alterations were found, mainly in chromosome regions 1p, 3p and 4p. Wu et al. (2006), using aCGH, found many chromosomal regions with genomic alterations that were shared between eutopic and ectopic endometria and suggested that these regions could harbor genes dysregulated both in ectopic and eutopic endometria from patients with endometriosis. The latest CGH study by Veiga-Castelli et al. (2010) included 10 women with ovarian endometrioma and revealed that although most chromosomal aberrations were shared between eutopic and ectopic endometria, some aberrations were seen only in the eutopic endometrium. On the contrary, Zafrakas et al. (2008), who studied endometriomas using aCGH found no genomic alterations in ectopic endometrial tissue.

SCNAs in the endometriotic foci have mostly been found when CGH or aCGH methods were used (Gogusev et al., 1999; Guo et al., 2004, Wu et al., 2006; Veiga-Castelli et al., 2010). Although CGH is a valuable method for discovering CNVs, it has been shown that some G−C-rich chromosomal regions (1p, 16p and chromosomes 19 and 22) tend to give false-positive results (Karhu et al., 1997; Veiga-Castelli et al., 2010). In addition, some genomic alterations reported in earlier studies (Guo et al., 2004; Wu et al., 2006) could be caused by whole genome amplification (WGA) used for multiplying the minute amounts of DNA obtained after LCM prior to CGH. Amplification artifacts generated by WGA can create false deletions and duplications and therefore it is not an ideal method for DNA amplification before CGH arrays (Talseth-Palmer et al., 2008). For SNP genotyping arrays, the amount of input DNA (200 ng) is much less than that required in CGH and therefore there is no need to use WGA for DNA obtained after LCM before using SNP arrays. Additionally, for methodological reasons, traditional CGH assays used in most studies do not provide data on absolute copy number of a given CNV, as the copy number of the reference sample DNA is unknown. Methodology used in our study eliminates inter- and intra-individual variability in DNA copy number alterations by comparing the CNV profile in blood to that in eutopic and ectopic endometria from the same individual.

As seen in Fig. 1, endometriotic lesion biopsy samples will contain variable amounts of surrounding tissue in addition to endometrial glands and stroma. Therefore, we used the LCM approach to harvest a population of purified endometriotic cells in an attempt to reveal the true genetic alterations characteristic of endometriotic lesions. We detected no SCNAs in ectopic or eutopic endometria and our results are in good agreement with those published by Zafrakas et al. (2008), who reported normal genomic patterns in all studied endometrioma samples. In our study four SCNAs with borderline confidence thresholds were called by one data analysis program or the other when DNA samples from LCM endometriotic foci were examined but none of these results were confirmed in a quantitative real-time PCR analysis. DNA extracted after LCM is usually fragmented (Aaltonen et al., 2011), which may produce some low-confidence alterations in array analysis. This accentuates the importance of using more than one program to analyze these DNA samples because none of these borderline confidence results were confirmed by both data analysis programs. However, all inherited CNVs were identified when LCM DNA was studied (Fig. 2), showing that DNA extracted after LCM can be used successfully for the detection of genetic changes by SNP arrays.

Nevertheless, as we studied only 17 endometriotic tissues derived from 11 patients we cannot entirely exclude the occurrence of rare SCNAs. Some existing SCNAs might be missed as probe distributions on SNP arrays depend on the availability of informative SNPs throughout the genome. That is particularly problematic for repetitive regions where only a few informative SNPs can be found. To overcome this problem the use of CNV-focused arrays containing dense probe tiling in known CNV regions is suggested (Haraksingh et al., 2011). Furthermore, it is possible that despite similar clinical, micro- and macroscopic appearances, different subtypes of endometriosis with diverse malignant potential exist as it has been shown that in some cases endometriosis could be a precursor for ovarian cancer (Bell, 2005).

Examination of DNA in blood, endometria and endometriotic foci of 11 patients showed the presence of 52 CNVs. Most of the CNVs found (in the blood, endometrial and endometriotic foci DNAs) are described in the Database of Genomic Variants as common CNVs and some of them were recurrent in our study group. The most frequent (45%) CNV in our study group was a homo- or hemizygous deletion in the 3p14.1 intergenic region downstream of *MAGI1*. The product of the *MAGI1* gene is a scaffolding protein at cell junctions, possibly inhibiting the migration and invasion of hepatocellular carcinoma via the regulation of the tumor suppressor gene PTEN (Zhang and Wang, 2011). Furthermore, Zaric et al. (2011) proposed that MAGI1 is a cyclooxygenase-2 IB induced inhibitor of the Wnt/b-catenin signalling pathway, with tumor-suppressive and anti-metastatic activity in experimental colon cancer.

Recent studies have suggested that besides SNPs, inherited CNVs significantly contribute to genetic predisposition to several common diseases (reviewed in Stankiewicz and Lupski, 2010). We hypothesized that a CNV in the downstream region of the *MAGI1* gene could influence MAGI1 expression and consequently be associated with genetic predisposition to endometriosis. Therefore, we decided to study this deletion frequency further in a larger sample of women, and our research revealed that homozygous and hemizygous deletions in this area were present at similar frequencies in patients with endometriosis

and control women without endometriosis. Although our control group consisted of healthy women with no history of endometriosis, we cannot entirely exclude the presence of undiagnosed asymptomatic endometriosis cases among the control women, as none of them had undergone diagnostic laparoscopy. Still, we believe that inclusion of some undiagnosed cases among the controls would have only a marginal effect on the 3p14.1 deletion frequency, with no effect on statistical significance. Thus, we propose that all common CNVs found in our study represent normal genomic variability and are not related to development of endometriosis. However, further studies including more patients are warranted to evaluate the possible role of common CNVs in genetic predisposition to endometriosis.

In addition to DNA copy number data, the determination of virtual karyotypes using SNP-based arrays also provides the possibility to detect areas of genomic LOH without loss of DNA, referred to as cn-LOH. The cn-LOH occurs when a segment of one chromosome is lost and replaced by the same region of its homologous chromosome leading to a homozygous state of point mutations or other microlesions in this region (reviewed in Heinrichs *et al.*, 2010). A wide range of cn-LOH regions have been found in breast and lung cancers and many other malignant conditions (reviewed in Tuna *et al.*, 2009). In our study, three cn-LOH regions (>5 Mb) present in all studied compartments (blood, eutopic and ectopic endometria) were seen. Two of them were in centromeric areas and one region was located at 18q21.1–21.3. We assume that these cn-LOH regions represent normal genomic variability, as they existed in all three studied tissues of a patient and no particular endometriotic foci-specific cn-LOH regions were found. Furthermore, regions with extended homozygosity of even >5 Mb have been reported in normal individuals (Simon-Sanchez *et al.*, 2007). However, the size of our study group was probably insufficient to definitely exclude associations between somatic cn-LOH regions and endometriosis, and additional studies are necessary.

In conclusion, our data provide evidence that no endometriosis-specific SCNAs or cases of cn-LOH are present in eutopic or ectopic endometria in our study group. Thus, molecular mechanisms other than chromosomal rearrangements most likely underlie the initiation and progression of this common gynecological disorder. A growing body of evidence indicates that epigenetic modifications rather than changes at the DNA level could be associated with the pathogenesis of endometriosis (reviewed in Guo, 2009). Nevertheless, sequencing of the whole genome from endometriosis lesions should be carried out to exclude the involvement of somatic gene mutations in the development of endometriosis.

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Authors' roles

M.S. was involved in conception and design of the study, performed the experiments and data analysis, interpreted the results and wrote the article. D.S. recruited the patients, collected medical data and prepared the manuscript. K.V. performed the experiments and edited the manuscript. P.P. performed bioinformatical data analysis. M.R. performed bioinformatical data analysis. M.L. assisted with laser-captured microdissection. H.K. collected patient's medical data and edited the manuscript. A.Sõ. recruited patients and collected tissue samples. A.Sa. was involved in conception and design of the study and edited the manuscript. T.D. was involved in co-writing and editing the manuscript. M.P. was involved in conception and design of the study, analyzed the data and prepared the manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

None declared.

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