DIFFERENTIAL GENE EXPRESSION OF EUTOPIC ENDOMETRIUM AND NORMAL PELVIC PERITONEUM IN WOMEN WITH AND WITHOUT ENDOMETRIOSIS

By

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NOMENCLATURE

3HM	3DNA hybridization Mix
AFS	American Fertility Society
AHR	Arylhydrocarbon receptor
AHRR	Arylhydrocarbon receptor repressor
AR	Androgen receptor
aRNA	Amplified Ribonucleic acid (RNA)
ARNT	Arylhydrocarbon receptor nuclear translocator
ASRM	American Society of Reproductive Medicine
CA-125	Cancer antigen-125
CAG	Cytosine, adenine, and guanine
cDHM	cDNA hybridization mix
cDNA	Complementary Deoxyribonucleic acid (DNA)
CGH	Comparative genomic hybridization
CNS	Central nervous system
CYP1A1	Cytochrome P450IA1
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiotreitol
dTTP	Deoxythymidine triphosphate
ECM	Extracellular matrix
ER	Estrogen receptor
EST	Expressed sequence tag
FISH	Fluorescence in-situ hybridization
GADD45B	Growth arrest and DNA-damage-inducible 45 beta
GAL	Genepix Array List
GALT	Galactose-1-phosphate uridyl transferase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GnRH	Gonadotropin-releasing hormone
GPAP	GenePix Autoprocessor
GSTM1	Glutathione S-transferase M1
GTSE1	G2 and S phase Expressed 1
IL-1	Interleukin-1
IL-1β	Interleukin-1beta
IL-6	Interleukin-6
KEGG	Kyoto Encyclopedia of Genes and Genomes
LH	Luteinizing hormone
LOH	Loss of heterozygosity
MAPK	Mitogen-activated protein kinase

MCM5	Minichromosome maintenance deficient 5, cell division cycle 46
MGB1	Mammoglobin
ml	Milliliter
MMP	Matrix metalloproteinase
ng	Nanogram
NO	Nitric oxide
NOS	Nitric oxide synthase
OVPG1	Oviductal glycoprotein 1
OXEGENE	Oxford Endometriosis Gene
PCR	Polymerase chain reaction
PPAR	Peroxisome proliferator-activated receptor
PR	Progesterone receptor
PROGINS	Progesterone receptor gene polymorphism
RNA	Ribonucleic acid
RT	Reverse transcriptase
RT-PCR	Reverse transcription-polymerase chain reaction
SOCS2	Suppressor of cytokine signaling 2
TGF-β	Transforming growth factor beta
THBS3	Thrombospondin 3
TNFSF12	Tumor necrosis factor superfamily, member 12
TNF-α	Tumor necrosis factor alpha
TNIP2	A20-binding inhibitor of NF-kappaB activation 2
Tob-1	Transducer of ErbB-1
TP53	Tumor protein p53
TWEAK	TNF-related weak inducer of apoptosis
β-ME	Beta-mercaptoethanol
μg	Microgram
μl	Microliter

CHAPTER I

INTRODUCTION

Endometriosis was first described in the scientific literature in 1860, by Austrian pathologist Karl Freiherr von Rokitansky, who referred to the disease in his writings as simply "an adenomyoma" (http://www.endocenter.org/womenshealthmarch.html). It was not until 1927 that John Sampson introduced the term “endometriosis,” postulating that retrograde menstrual flow transported desquamated endometrial cells into the peritoneal cavity, and that viable cells subsequently implanted and grew (1). His theory has remained paramount, however other theories, including coelomic metaplasia, exposure to toxins, and genomic alterations, are believed to have roles as well. Although endometriosis has remained an enigmatic disease, there has been an explosion in clinical and basic science information in the last five years, with research predominantly in the disciplines of genetics, environmental science, cancer biology, and immunology (2).

It is likely that heritable genetic factors contribute to the development of endometriosis. Although the genetic risk may be due to a single gene, it is probably due to activation of a number of genes, each responsible for a small incremental increase in risk (3). Several large-scale studies aimed at identifying disease susceptibility genes are currently underway. Both the Oxford Endometriosis Gene (OXEGENE) study and an Australian collaborative study are using affected sibling pairs and positional cloning

techniques to identify regions of chromosomes that may harbor susceptibility genes for endometriosis (4).

The investigation of gene expression in the past has been limited by the inability to study more than one gene at a time. The introduction of deoxyribonucleic acid (DNA) microarray technology has made it possible to examine the expression of thousands of genes at a time. Recently, DNA microarray analysis was found to be an effective tool for the identification of differentially expressed genes between uterine and ectopic endometrium. This study found that the expression of eight genes, from a total of 4,133 genes on the DNA microarray, was increased in endometriotic implants compared with uterine endometrium (5). However, no endometrial samples were tested from patients who did not have endometriosis. If the uterine endometrium of patients with endometriosis exhibits defective or altered gene expression, which is subsequently involved in the ability of endometrial tissue to implant in the abdomen, then this study would have missed that gene expression change.

Another recent study utilizing DNA microarray technology compared gene expression in the eutopic endometrium of women with or without endometriosis. This group utilized a human microarray containing with a total of 9,600 genes. While a difference in gene expression was found between the two types of eutopic endometrium, the study did not test any endometriotic tissue (6). As a result, there is still a need for gene expression analysis involving normal eutopic endometrium in women with and without endometriosis, as well as of ectopic endometriotic lesions throughout all stages of endometriosis.

Women with endometriosis have significantly more peritoneal macrophages and oviductal macrophages compared to normal fertile controls. Peritoneal macrophages

from women with endometriosis demonstrate increased phagocytosis, secretion of cytokines, prostaglandins, growth factors, and enzymes (7). Peritoneal fluid containing immune-related cells is often seen in the vesicouterine cavity or pouch of Douglas during gynecologic surgery and bathes the pelvic cavity, uterus, fallopian tubes, and ovaries. It may be a major factor controlling the peritoneal microenvironment, influencing the development and progression of endometriosis and endometriosis-associated infertility (8). Peritoneal macrophages have reduced capacity to destroy endometrial cells and ectopic endometrial cells have increased resistance to macrophage-mediated cytolysis. The many growth factors secreted by macrophages in patients with endometriosis provide a vehicle by which endometrial cellular proliferation can be promoted (9). Peritoneal macrophages from women with endometriosis also have increased ability to phagocytize and kill normal sperm in vitro, due in part to increased concentrations of nitric oxide (NO). The presence of NO-producing peritoneal macrophages in women with endometriosis may be one reason for reduced fertility in this disease (7). The direct effects of peritoneal macrophages on endometriotic implant development and infertility have yet to be elucidated. Examination into the differential gene expression of peritoneal macrophages in women who have developed endometriosis versus those who do not exhibit disease would assist in increased understanding of macrophage mechanisms of action in this disease.

Statement of the Problem

To observe the differences in gene expression in eutopic endometrial tissue and normal pelvic peritoneum in women with and without endometriosis, and to observe the

differences and/or possible progression in gene expression throughout all four stages of endometriosis.

Purpose of the Study

The purpose of this study was to conduct a more thorough examination of gene expression in women with and without endometriosis than has been performed to date. This allows for a more complete identification of the genes up-regulated and down-regulated in the eutopic endometrium and normal pelvic peritoneum of women with endometriosis versus those without.

Significance of the Study

The benefits of this study include gaining a deeper knowledge of the genetic basis of endometriosis. Previous studies have already discovered that there is a difference in gene expression between normal endometrium and ectopic tissue in women with endometriosis, as well as a difference in eutopic endometrium of women with and without endometriosis. This study's aim was to provide analyses not only on a larger scale (i.e. more normal subjects and diseased patients), but also using human cDNA microarrays that will allow for inspection of approximately 23,000 genes. This will allow for a greater understanding of how gene expression affects disease expression, which may lead to subsequent studies of specific genes that have a role in the pathogenesis of endometriosis. Studies such as this one may help in predicting and potentially preventing disease development and/or progression in women with a family history of disease and may lead to screening practices for all women. In addition, this

information could lead to more specific treatments, including refined surgical procedures, pharmaceutical regimens, and other therapies designed for patients suffering from endometriosis and associated pelvic pain and infertility.

CHAPTER II

REVIEW OF LITERATURE

Endometriosis: The Disease

What is Endometriosis?

Endometriosis is defined as the presence of endometrial cells and stroma outside the uterine cavity. It is one of the most common gynecologic diseases, with an incidence of approximately 10% to 15% of women of reproductive age (10, 11) and 21% to 44% of infertile women (7). It is observed in up to 25% of all gynecological laparoscopies and laparotomies (12).

Although the numbers differ among studies, a recent examination of the frequency of sites of endometriosis found that the two most common areas were the uterosacral ligaments (63%) and the superficial part of the ovary (56%), with less incidence involving the ovarian fossae (32.5%), anterior vesical pouch (21.5%), ovarian endometriomas (19.5%), pouch of Douglas (18.5%), broad ligament (7.5%), intestines (5%), uterus (4.5%), and fallopian tubes/mesosalpinx (4%) (13).

It is not possible to know the exact prevalence of endometriosis, as it can only be definitively diagnosed via diagnostic laparoscopy. At this time, prevalence has only been assessed based on patient presentation. This has shown a 7% prevalence in women with a first-degree relative with endometriosis, as well as a 15% to 25% prevalence in infertile women (13). One population-based study found a prevalence of 6.2% in the general

female population (14), however, other studies have suggested this is low, with anywhere from 8% to 15 % of women having the disease. Its prevalence is considered to be 47% to 65% in women under age 20 who have chronic pelvic pain and/or dyspareunia (15).

Clinical Presentation of Endometriosis

The most common symptoms associated with endometriosis are dysmenorrhea, dyspareunia, chronic pelvic pain, and infertility. There is a poor correlation between symptoms and severity of disease; women with extensive endometriosis may be asymptomatic and their endometriosis found incidentally upon laparoscopic surgery for infertility or another issue. In the same fashion, women with minimal or mild disease may present with severe symptoms (10).

Symptoms of endometriosis have been associated with the location of endometriotic lesions. Dysmenorrhea, lower abdominal, pelvic and back pain, dyspareunia, infertility, menstrual irregularity, and acute pelvic pain due to rupture or torsion of an endometrioma can all be associated with endometriotic implants in the female reproductive tract. Endometriosis in the gastrointestinal tract can be associated with cyclical tenesmus, rectal bleeding, diarrhea, and colonic obstruction. Cyclical hematuria and pain, as well as ureteral obstruction have been linked with endometriosis within the urinary tract. Pain and bleeding in surgical scars and in the umbilicus, as well as hemoptysis of the lung in a cyclical fashion have all been symptoms of endometriosis within those regions (13).

Types of Endometriosis

Over fifteen laparoscopic appearances of peritoneal endometriosis have been described, including classical, vesicular, popular, hemorrhagic, nodular, and healed implants, with histologic studies leading to three further subdivisions: free, enclosed, and healed implants (13). According to the most recent correspondence on the American Society of Reproductive Medicine (ASRM) classification system, the morphology of peritoneal and ovarian lesions should be categorized as red (red, red-pink, and clear lesions), white (white, yellow-brown, and peritoneal defects), or black (black and blue lesions) (16).

Red lesions are more active forms of disease. Red lesions demonstrate an increased level of vascularization, caused by a larger number of larger vessels. White lesions have a greater number of smaller vessels, including an increased number of capillaries as compared to red lesions. Red, flame-like lesions are theorized to be representative of an early stage of endometriotic implantation. Menstrual shedding from viable implants could initiate an inflammatory reaction and the scarification process. The implant is subsequently enclosed and the presence of intraluminal debris leads to the typical blue or black coloration. Sometimes these lesions encase bleeding tissue, which can contribute a brown pigment as well. If the lesions are puckered, they may represent a combination of glands, stroma, and luminal debris (17). The black lesions were at one time considered the premier visual indication of endometriosis, due to older studies characterizing endometriosis as a disease of women in their mid-to-late thirties, which is when this stage of lesion is most typically found (18). The inflammatory process continues and devascularizes the implant, and white plaques of old collagen remain. White lesions typically represent a latent stage of endometriosis that is non-active (17).

Another category of endometriosis frequently referenced in the literature is an endometrioma or chocolate cyst. An endometrioma is an endometriotic cyst of the ovary. The term "chocolate cyst" developed due to the dark brown/chocolate-colored contents of these ovarian cysts when aspirated during laparoscopic surgery (13) or via ovarioscopy (19). Cystic ovarian endometriosis is often hard to distinguish from a corpus luteum cyst. One method of distinguishing them, alongside visualizing the contents of the cyst, is recognition of adhesions. Endometriotic ovarian cysts typically have associated adhesions, especially in the ovarian fossa, whereas a corpus luteum cyst typically does not (19).

ASRM Classification

Several classification schemes have been developed over the years to categorize the severity of endometriosis. As early as 1921, Sampson classified endometriosis by modifying a system used to categorize hemorrhagic cysts of the ovary. A method developed by MJ Wicks and CP Larson in 1949, borrowed from a system Broder used to stage malignancy, classified lesions as Grade I-IV, based upon histologic examination of excised lesions (20). Linking endometriosis with malignancy, however, led to a treatment of hysterectomy with bilateral salpingo-oophorectomy for the majority of patients, including adolescents and teenage women (21).

Until the 1970's, no other system was proposed for classifying endometriosis (21). During that time, a few systems were put forward, including Acosta's, which classified endometriosis into mild, moderate, and severe categories based upon site of lesions, presence of adhesions, and presence of scarring (13, 21, 22). Another system, developed by Kistner in 1977, incorporated progression of endometriotic disease (13, 21,

23). This was followed by Buttram's classification, which was considered to be an expanded version of Acosta's, and graded patients by peritoneal, ovarian, tubal, and cul-de-sac involvement (21, 24). While Acosta's system had seemed too vague, Buttram's was considered too complicated, therefore another system was proposed by Cohen which divided severity into ten states based on laparoscopic findings, including distant organ involvement, adenomyosis, and pelvic inflammatory disease (21, 25).

The American Fertility Society (AFS, renamed the American Society for Reproductive Medicine in 1995) formed a committee to establish a classification system for endometriosis in 1978. A revised version of this classification, published in 1996, is the classification system currently utilized by obstetricians and gynecologists. The ASRM classification enables a physician to determine the stage of endometriosis utilizing a weighted point system. Endometriosis, posterior cul-de-sac obliteration, and adhesions are evaluated visually and then assigned points based on superficial or deep involvement of the peritoneum and/or ovaries (endometriosis), partial or complete obliteration (posterior cul-de-sac), and filmy or dense enclosure of the ovaries or fallopian tubes (adhesions). The appearance of superficial implants is also recorded as red, white, or black, with the percents of each determined and equaling 100%. Endometriosis visualized in other locations is recorded under "additional endometriosis," and other pathology documented under "associated pathology" with accompanying sketches. The accumulation of all points will determine the stage of endometriosis, with the point distributions as follows: Stage I (Minimal), 1-5; Stage II (Mild), 6-15; Stage III (Moderate), 16-40; and Stage IV (Severe), >40 (16).

Criticism of the ASRM classification system has included arbitrariness in both the assignment of points, depending upon the location of an endometriotic lesion, as well as

in the lack of distinguishing characteristics between stages. It is also very important that physicians staging endometriosis have experience in utilizing the system and are consistent in their diagnoses. Reproducibility has been questioned and examined, looking at both intraobserver and interobserver variability in staging endometriosis. Prior to the most recently revised ASRM classification of endometriosis, a study looking at the reproducibility of the 1985 revised AFS endometriosis classification system found a fair to good reproducibility ($\kappa = 0.44$) that was statistically significant ($p < 0.01$), when comparing assignments to disease stage made by investigators followed by blinded reviewers (26).

Another, more recent study examined the reproducibility of the revised AFS classification scheme when a physician scored the same patient twice, first by laparoscopy and then laparotomically during the next cycle. The inter-method variation altered endometriosis staging in 34.5% of patients, with 3.6% of patients' changed by two stages. The kappa coefficient indicated fair-to-good agreement between the two scoring methods ($\kappa = 0.49$). The predominant area of variability in scoring involved the ovaries, and this was believed to be due to assessment of some small endometriomas as ovarian cysts (27).

The correlation between histologic and visual diagnosis has also been investigated. Of 44 patients evaluated, visual AFS scores were an average of 4.0 points higher than histologic scores, with the visually-determined stage being higher in 36% (16) of patients, including 12 whose diagnoses were downgraded from stage I, II, or III to zero (28).

A better classification system for endometriosis is needed. Incorporating factors beyond visual assessment of the disease will be beneficial in designing a system that can

better delineate between stages of the disease and help in guiding treatment. Revisions to the revised ASRM classification are anticipated as our knowledge of endometriotic disease and its relationship to pelvic pain and infertility expands (21).

Tissues within which Endometriosis Has Been Found

Endometriosis has been classified as direct/internal/endometriosis interna or indirect/external/endometriosis externa (13, 29). In regard to external endometriosis, this category has been further classified into pelvic and extrapelvic. Pelvic endometriosis consists of endometriosis found on the uterus, fallopian tubes, ovaries, and pelvic peritoneum (anterior and posterior cul-de-sacs and pelvic side walls). Extrapelvic endometriosis refers to the presence of endometriotic tissue anywhere else (29).

Endometriosis has been documented in almost every organ system and tissue in the body. Endometriosis has been found in the central nervous system (CNS), lungs, pleura, heart, diaphragm, gallbladder, liver, small bowel, appendix, colon, rectum, kidney, bladder, ureter, bone, biceps muscle, and the sciatic nerve (29, 30). In addition, endometriosis has been found in the umbilicus, abdominal wall incisions, including Cesarean section scars, and episiotomy scars (13, 29).

The gastrointestinal tract is the most common site for extrapelvic endometriosis. Women in their thirties are most commonly affected; however, gastrointestinal endometriosis is usually asymptomatic and considered to be clinically unimportant. The rectum and the sigmoid colon are the most common sites, representing up to 95% of cases. The appendix is involved 5-20% of the time, and the small bowel is implicated in less than 5% of cases, with the terminal ileum being the most common site.

Endometriosis of the transverse colon is rare, as is that of the gallbladder, liver, and pancreas (29).

Urinary tract endometriosis was first published in 1921, and only a few hundred cases have been reported since. One to four percent of women with pelvic endometriosis have urinary tract endometriosis too. Major morbidity includes functional loss of the kidney, if endometriosis obstructs a ureter. The bladder is the most common site of urinary tract endometriosis, comprising 80-90% of cases. Renal endometriosis is rare, with less than a dozen cases reported, as is urethral endometriosis. Typically, unilateral disease is found, with the left side more often than the right, although bilateral disease does occur (29).

Just over one hundred cases of thoracic endometriosis have been reported. Fifty to eighty percent of women with thoracic endometriosis also have pelvic endometriosis. Thoracic endometriotic lesions are usually solitary and involve the pleura or the lung parenchyma. The right side is implicated more often than the left (9:1), however bilateral diagnoses have been reported. Signs and symptoms of thoracic endometriosis include pneumothorax, followed by hemothorax, hemoptysis, or an asymptomatic lung nodule, with concurrent chest pain. Unlike other forms of pulmonary pathology, the signs and symptoms of endometriosis in the thoracic area are generally cyclical, occurring within 24 to 48 hours of the onset of menses, and the lesions enlarge at this time (29).

Extrapelvic endometriosis has also been found in other tissues of the body, most commonly in cutaneous tissues, but also in the central and peripheral nervous systems, as well as isolated cases in bone and skeletal muscle. Cutaneous development of endometriosis is frequently at sites of previous abdominal operations, with signs and symptoms beginning six months to a few years after surgery. Endometriosis in Cesarean

section scars and episiotomy scars has also been found, as well as in the inguinal area, labia, perineum, and vagina. Umbilical endometriosis has developed de novo, as well as following laparoscopic tubal ligation. Endometriotic lesions have also been found in abdominal wall trocar tracts, and the incidence of this is expected to grow with increased use of laparoscopic surgical techniques. In addition, endometriosis has been found in an amniocentesis needle tract. Signs and symptoms of cutaneous endometriosis include a slowly growing, tender mass that may increase in size and pain during menses. Cyclical bleeding at the site of the lesion may also be present (13, 29).

In addition to extrapelvic endometriosis, endometriotic lesions have also been found in men. Although the pathogenesis remains unexplained, all cases have involved men with prostate cancer being treated with excessive estrogen therapy. Endometriosis has been found on the right side of the bladder, between the trigone and the domes, on the left side of the trigone, and obstructing the right ureter at the right ureterovesical junction, producing moderate hydronephros. Although none of these lesions were found near the prostatic utricle, which alongside the appendices testes is considered to be a remnant of the mullerian duct in men. It is believed, however, that some vestigial endometrial glands may persist in males (31).

Methods of Diagnosing Endometriosis and Diagnostic Delay

The “gold standard” for diagnosing endometriosis is visualization by laparoscopy (32). There is no question that the development of a laboratory test to diagnose endometriosis would be invaluable. Great efforts have been made to discover a non-invasive method of diagnosis, such as a serum marker. One example is cancer antigen-125 (CA-125), which is present in fetal coelomic epithelium and its derivatives such as

pleura, pericardium, peritoneum, ovarian surface epithelium, and mullerian ducts after 15 weeks' gestation. It becomes strongly positive in mullerian-derived epithelia of adult, such as tubal, endometrial, and endocervical epithelium (33). Serum interleukin-6 (IL-6) and peritoneal fluid tumor necrosis factor-alpha have also been shown, preliminarily, to discriminate between women with and without endometriosis (34).

A major problem in endometriosis is diagnostic delay. Studies surveying the United States, United Kingdom, and Norway have found that there is considerable delay in the diagnosis of endometriosis. It is estimated that 27% of U.S. women diagnosed with endometriosis have had symptoms for over 6 years (13).

Pharmaceutical and Surgical Treatment of Endometriosis

Oral contraceptive pills are typically the first-line agent utilized in treating pelvic pain suspected of being endometriosis. Although oral contraceptives have been promoted as a possible means of protection from endometriosis, if they are used noncontinuously and menstruation is allowed to occur, then blood would still be retrograded into the pelvis. Therefore, continuous administration may be necessary to truly provide protection (35). Oral medroxyprogesterone acetate (Provera) is the most common progestational agent used to treat endometriosis, with typical doses up to 50-100 milligrams per day (36). GnRH agonists, available in the United States since 1985, include leuprolide acetate (Lupron), nafarelin acetate (Synarel), and goserelin acetate (Zoladex) (37). They facilitate the healing process by hormonal inactivation of endometriotic tissue. Another pharmaceutical method of treating endometriosis is danazol, which suppresses pituitary secretion of gonadotropins and inhibits ovulation, but has a high incidence of side effects including weight gain, hirsutism, liver damage, and

irreversible voice deepening (36, 38). A couple of prospective treatments include aromatase inhibitors and pentoxifyllin, an anti-tumor necrosis factor therapy (38, 39).

Surgical treatment of endometriosis can be conducted on many levels, including minimalistic, conservative, semi-conservative, radical, or definitive. Laparoscopic surgical treatment may include bipolar or thermal coagulation and vaporization, excision of lesions with a carbon dioxide laser or unipolar knife, presacral neurectomy, and uterine nerve ablation (40).

From 1988-1993, 214,059 hysterectomies were performed in the United States on women 25-29 years old, including 64,883 performed for the primary diagnosis of endometriosis (41). Hysterectomy with or without bilateral salpingoophorectomy is often reserved for those patients with intractable pain who have failed medical and conservative surgical therapies. Typically, patients choose this type of procedure only after childbearing has been completed. However, some women must make such a choice at a young age in hope of permanent cure (41).

Theories of Disease Pathogenesis

Numerous theories have been proposed to explain the pathogenesis of endometriosis. Some hypotheses are based on endometriotic tissue originating from the eutopic endometrium, while others are based on tissue originating from somewhere else. The most popular theory, implantation following retrograde menstruation, was proposed by Sampson in the 1920s (42). According to this hypothesis, endometriosis is established during retrograde menstruation, when menstrual fragments flow out of the fimbriated ends of the fallopian tubes and implant on the ovary and other peritoneal sites. Internal

bleeding in ovarian lesions can result in chocolate cysts. Repeated retrograde menstruation can lead to the spread of numerous endometriotic implants throughout the pelvic cavity. Endometriotic lesions are presumed to bleed periodically and stimulate inflammatory processes that lead to adhesions between the uterus, ovaries, and pelvic viscera. These lesions regress during menopause and the condition is generally considered to be an estrogen-dependent disease.

Other theories assuming the origin of endometriotic tissue to be eutopic endometrial cells, include the direct-extension theory, the benign metastasis theory, the uterotubal theory, and mechanical transplantation. The Wolffian duct theory, the Mullerian duct theory, the coelomic metaplasia theory, the hormonal stimulation theory, and the induction phenomenon theory are all based on the endometriosis developing from tissues other than eutopic endometrium (43).

Retrograde Menstruation

Retrograde menstruation involves the reflux of endometrial tissue through the fallopian tubes during menstruation and subsequently implanting on the pelvic organs and/or the peritoneum. Three assumptions are necessary to support this theory. First, retrograde menstruation occurs. Second, upon being refluxed through the fallopian tubes, endometrial cells are viable in the peritoneal cavity. Lastly, endometrial cells refluxed through the fallopian tubes have the ability to adhere to the peritoneum and then invade, implant, and proliferate (44). In a study by Halme et al., 90% of normal and infertile women were found to have blood in their peritoneal fluid during the perimenstrual (days one to six or 27 to 30) portion of their menstrual cycle (35).

However, arguments have been made against Sampson's theory. Redwine compared the implantation of endometrial cells following retrograde menstruation, with other types of autotransplants. He found that endometriotic tissue is not the same as eutopic endometrium, therefore, it lacks characteristics of an autotransplant. He further stated that studies of experimental endometriosis that have not used menstrual endometrium may be misleading (45). In an editorial follow-up to this study, Guo critiqued Redwine's conclusions on several counts. Guo felt that the differences found between eutopic and ectopic endometrium could be due to changes experienced by cells that have successfully implanted and undergone subsequent clonal expansion and inflammation. He also criticized Redwine's dismissal of endometrial cell damage within the fallopian tube, stating that due to the enormous turnover of cells due to monthly shedding, it is certainly possible for genomic alterations of cells to occur that might enable cells to implant and lead to endometriosis. Guo referred to the loss of heterozygosity and other genomic alterations that have been found in the cells of endometriotic implants (46).

Coelomic Metaplasia

The notion that "endometrium begets endometriosis" has been used to support the retrograde menstruation and transplantation theory of endometriotic pathogenesis. However, a case involving the discovery of ovarian and tubal endometriosis in a 26-year-old woman without the presence of eutopic endometrium has been reported (47). A similar situation was seen in a young girl in whom endometriosis was found developing within the left cornu of a bicornuate uterus, but associated pelvic pain had been experienced in a cyclical nature eight months prior to menarche (48).

The coelomic metaplasia theory was introduced at the turn of the century by Iwanoff and Meyer (13, 44, 49). It proposed that endometriosis develops from metaplasia of cells that line the pelvic peritoneum (13, 49). The development of endometriosis in men is typically thought of as proof of the coelomic metaplasia theory. However, the men within which endometriosis was found were undergoing estrogen therapy, and the possibility of estrogen stimulation of müllerian rests cannot be excluded. Pleural endometriosis could result from local metaplasia of pleural mesothelium, but it might result from transdiaphragmatic passage of endometrial fragments (44). If this theory is correct, metaplasia should occur in other regions where coelomic membranes are present, and an increased frequency would be expected with aging (13, 44). However, endometriotic disease is typically present in women of reproductive age, with a dramatic decrease in prevalence as women become postmenopausal (13). Therefore, proof of the coelomic metaplasia theory is inconclusive (44).

Endometriosis found in the extremities has also been cited in support of the coelomic metaplasia theory of endometriosis. Misintegration of coelomic membrane-related cells into the limb buds has been proposed, due to the fact that mesenchymal limb buds develop next to coelomic epithelium during early embryogenesis. These cells could then be stimulated by ovarian steroids and develop into endometriosis (49).

Induction Theory

The induction theory is an extension of the coelomic metaplasia theory. It was introduced by Levander and Normann in 1955 (50). This theory proposes that endogenous biochemical or immunologic factors of menstrual effluent can induce undifferentiated cells to differentiate into endometrial tissue (44, 51).

Embryonic Rest Theory

Von Recklinghausen and Russell introduced the embryonic rest theory in the 1890s. The embryonic rest theory proposed that cell rests of müllerian origin could be activated to differentiate into endometrium in the presence of a specific stimulus. The transformation of embryonic rests is considered to be a possible explanation for the rare cases of endometriosis reported in males (44).

Lymphatic and Vascular Metastasis Theories

The theory that endometriosis could result from lymphatic and hematogenous dissemination of endometrial cells was proposed by Halban and Sampson in the 1920s. It had been found that endometrial cells could metastasize via lymphatic and hematogenous routes (44). In an original communication by Sampson, he wrote:

> "Could bits of endometrial tissue escape into the venous circulation from the mucosa lining the uterine cavity by any other means than as a result of a menstrual reaction? . . . We could also understand that a similar endometrial lesion might rupture into an adjoining lymph vessel or capillary during its reaction to menstruation . . . I can see only one correct interpretation of the etiology of the embolic endometrial lesions in the veins about these endometrial cavities and that is they arose from the implantation of endometrial tissue, disseminated into the veins from the menstrual rupture of the walls of the endometrial cavities into these vessels" (42).

Metastasis of endometrial cells through the lymphatic system to distant areas, such as pleura, umbilicus, retroperitoneal space, lower extremity, vagina, and cervix is anatomically possible because of communication of lymphatics among these structures. Lymphatic or vascular metastasis could explain rare cases of endometriosis that have been reported in bone, muscle, brain, nerve, lung parenchyma, vertebral space, and

extremities (44). Lymphatic spread has also been thought to explain the presence of endometriosis in lymph nodes (33).

Iatrogenic Dissemination

Endometriosis has developed due to iatrogenic transplantation of endometrial cells during gynecologic surgeries and obstetrical procedures. It has been found in abdominal wall scars after Cesarean sections, myomectomies, and hysterectomies, as well as within episiotomy scars (13).

Steps in Pathogenesis

The steps involved in the pathogenesis of peritoneal endometriotic implants include: (1) attachment of endometrial cells to peritoneal surface; (2) invasion of endometrial cells into mesothelium; (3) angiogenesis around nascent endometriotic implants; (4) endometrial cell proliferation; and (5) recruitment of inflammatory cells subservient to the implant (44, 52).

Relationship between Endometriosis and other Disease

Autoimmune disease

The presence of endometriotic tissue has long been thought to involved disruptions in the immune system or surveillance. One study, conducted by the Endometriosis Association looked at the rates of comorbidities of autoimmune, endocrine, and chronic fatigue or pain states in women with surgically diagnosed endometriosis. Of the 3680 respondents included in the analyses, approximately 20%

had at least one co-existing disease. The percentage of women with endometriosis with certain disorders versus the estimated prevalence of these disorders in the general U.S. female population were as follows: Systemic lupus erythematosus (0.8 versus 0.04%), multiple sclerosis (0.5 versus 0.07%), rheumatoid arthritis (1.8 versus 1.2%), Sjogren's syndrome (0.6 versus 0.03%), hypothyroidism (9.6 versus 1.5%), fibromyalgia (5.9 versus 3.4%), and chronic fatigue syndrome (4.6 versus 0.03%). All percentages were greater in women with endometriosis at a high level of significance ($p < 0.0001$), except for rheumatoid arthritis ($p = 0.001$) (53).

Cancer

Sampson hypothesized that endometriosis sometimes undergoes malignant change. Although usually benign, endometriosis exhibits some characteristics reminiscent of malignancy, including local invasion and metastases. Endometriosis and ovarian cancer share many risk factors, including early menarche, regular periods, shorter cycle lengths, and lower parity. There have been a number of studies investigating the association between endometriosis and ovarian cancer (54-58).

One of the most common sites of endometriosis is the ovary. The first molecular genetic evidence that at least a subset of endometriotic lesions may be premalignant came from an analysis for loss of heterozygosity (LOH) at candidate tumor-suppressor gene loci in a series of 40 endometriosis cases. Fifteen microsatellite markers were used to detect LOH for chromosome regions 6q, 9p, 11q, 17p, 17q, and 22q. These regions were selected because they were thought to harbor tumor-suppressor genes involved in the development of ovarian and other epithelial cancers. Eleven of 40 cases (27.5%)

demonstrated LOH of 9p, 11q, and 22q. In the endometrioid ovarian carcinomas, LOH was seen on 6q, 9p, 11q, 17p, 17q, and 22q (12).

A follow-up to this study analyzed genetic alterations present in endometriosis synchronous with ovarian cancer, to determine whether cancer had arisen de novo or by clonal expansion from endometriosis. Fourteen cases of ovarian endometriosis synchronous with ovarian carcinoma were analyzed. LOH analysis was performed using 25 microsatellite markers for chromosome regions 2q, 4q, 5p, 5q, 6q, 7p, 9p, 11q, 17p, 17q, 22q, and Xq. Five of seven cases with carcinoma adjacent to endometriosis displayed common LOH events. All four cases with carcinoma arising within an endometriotic cyst displayed at least two LOH events in common. None of the three cases where endometriosis and cancer were from contralateral ovaries displayed any common LOH. LOH was only detected for 5q, 9p, 11q, and 22q among solitary endometriosis cases. LOH was also detected at these loci in the endometriosis cases with associated carcinoma. The chromosome regions showing the highest LOH frequency among endometriosis cases (5q, 9p, 11q, 22q) were among the most frequent LOH regions in low-grade and early-stage endometrioid ovarian cancer. This data was considered support for the view that endometriosis could undergo malignant transformation and implied that tumor-suppressor gene alterations are likely to be involved in the proliferation and maintenance of endometriotic implants (59).

Endometriosis and the Peritoneal Environment

Mechanisms must exist that promote endometrial cell adhesion to the peritoneum and their subsequent survival and proliferation. Shed endometrial cells are removed by immune cells in the peritoneal cavity (e.g. macrophages). The fate of endometrial cells

that elude this process may be modulated by adhesion molecules expressed on their cell surface. Adhesion could also be favored if the peritoneal surface is damaged by trauma, low-grade inflammation, infection, or excessive retrograde menstruation. However, small implants are now considered a variant of normal physiological processes. For these to progress to endometriosis, they must undergo cellular modification, growth, and vascularization, all of which are promoted by hormones, growth factors, and cytokines (4).

Macrophages constitute 85% of the cells in the peritoneal fluid. Their number and level of activation varies over the course of the menstrual cycle, with a maximum in the post-menstrual period for removal from the peritoneal cavity of endometrial debris, spermatozoa, and follicular cells. In endometriosis patients, peritoneal macrophages are more numerous and have a higher level of activation (60).

The peritoneal fluid of endometriosis patients contains chemotactic cytokines, originating from T-cells, macrophages, mesothelial cells, and ectopic endometrium. The endometrium of endometriosis patients liberates only small amounts of cytokines that regulate the activation of macrophages, in contrast to the endometrium of unaffected women. The cytotoxicity of peritoneal macrophages in endometriosis patients with respect to the endometrium is reduced which could be more significant than that of the circulating macrophages. The alteration of the cytotoxicity could be specific to the endometrium be it eutopic or ectopic, since the macrophages of endometriosis patients normally destroy the usual target cells of macrophages (60).

Macrophages are primarily responsible for the phagocytosis of cellular debris, including sperm, in the pelvis. In a study by Muscato et al. (61), peritoneal macrophages phagocytized sperm in vitro and were more active in women with endometriosis than

those in women without the disease. Therefore, peritoneal fluid diffusing into the tubal and endometrial environment may affect sperm and their interaction with the oocyte (8).

Freshly isolated peritoneal macrophages from infertile women with endometriosis, compared to those from normal fertile women, have higher nitric oxide synthase (NOS) activity and produce more nitric oxide (NO) in vitro (7). A strong correlation has been found between the number of peritoneal macrophages in endometriosis patients and the total amount of peritoneal fluid NO. In addition to increased peritoneal fluid volume and macrophage number, women with endometriosis have significantly more oviductal macrophages than women without endometriosis. The oviduct lumen is contiguous with the peritoneal cavity, and peritoneal macrophages in endometriosis emigrate into the oviducts, resulting in elevated numbers of oviductal macrophages (7).

The presence of NO-producing peritoneal macrophages in the oviducts of women with endometriosis may be a reason for reduced fertility in the disease. At low concentrations, NO enhances sperm motility and capacitation. High concentrations of NO inhibit sperm motility, respiration, and viability. NO may also display toxic effects on the oocyte and embryo. Low levels are important in ovarian function and implantation and cause relaxation of oviduct muscles. Development of both murine and bovine embryos in vitro is inhibited by high levels of NO. In addition to its effects on reproduction, peritoneal NO may serve as a mediator of inflammation and be a major determinant of the sterile peritoneal inflammation seen in women with endometriosis (7).

Genetic Studies involving Endometriosis

Since the 1940s, published reports have recorded multiple affected relatives with endometriosis, often suggesting genetic tendencies. Epidemiological findings have shown familial tendency with increased risk (6.9%) and severity of endometriosis in first degree relatives of probands (62). Studies of twins have also suggested that endometriosis may have a genetic basis, including a recent study on 3,096 twin sister pairs, 215 of whom reported endometriosis, a prevalence rate of 0.07. In addition, concordance in monozygotic twins exceeded that in dizygotic twins by a factor of 2 (63). However, until genetic markers are determined, it is difficult to counsel individuals that have a first-degree relative with endometriosis.

Two forms of genetic mutations are germ line and somatic. Germ line mutations are inherited mutations affecting all cells, while somatic mutations involve genetic damage due to extrinsic or intrinsic factors, which may be exacerbated by inherited mutations. Somatic mutations affect only one cell type. Molecular genetic defects in endometriosis do not affect all cells, therefore, endometriosis is believed to be the result of somatic cell damage (64).

Polymorphism Studies

Several polymorphism studies have been done within the last decade involving endometriosis. In 2001, an analysis of the human arylhydrocarbon receptor repressor (*AHRR*) gene polymorphism was performed. Although a novel polymorphism was found, involving a substitution of alanine by proline at codon 185 in exon 5 of the *AHRR* gene, no significant difference in the frequency of genotypes or alleles was observed between women with and women without endometriosis. In addition, differences in the

frequencies of genotypes for the arylhydrocarbon receptor (*AHR*), as well as differences in allelic frequencies of known single nucleotide polymorphisms in *AHR*, aryl hydrocarbon receptor nuclear translocator (*ARNT*), and the cytochrome P450IA1 (*CYP1A1*) promoter were examined in women with and without endometriosis, with no significant differences found. The researchers felt that a subsequent study with a larger group of subjects would be necessary to determine whether polymorphisms within the *AHR* gene or the *AHRR* gene affect susceptibility to endometriosis (65).

Another group studied the p53 codon 72 polymorphism in Chinese women. They examined the distributions of p53 polymorphisms in women with and without endometriosis finding that the p53 polymorphisms differed significantly between groups ($p < 0.001$). In women with endometriosis, Arg/Arg homozygotes were represented in 10.2% of the population, with Pro/Pro being 22.9%, and Arg/Pro 66.9%. This differed from women without endometriosis, with 30.7%, 19.3%, and 50%, respectively. The Arg polymorphism was more common in normal women versus more Pro homozygotes and heterozygotes among the women with endometriosis. The researchers believed that p53 polymorphisms might be a useful marker in predicting endometriosis development, since, based on their study population, the odds that a woman with Arg/Pro or Pro/Pro will experience endometriosis was 3.9 times greater than that of a woman with the Arg/Arg genotype (66).

Recently, a second group examined the prevalence of p53 codon 72 polymorphisms in a Japanese population. The frequencies of the Arg/Arg, Arg/Pro, and Pro/Pro genotypes in endometriosis versus controls were 35.2% vs. 39.4%, 48.6% vs. 41.7%, and 16.2% vs. 18.9% indicating no significant difference in the frequency of p53 codon 72 polymorphisms. Upon examining a subgroup of women with stage IV disease

only, the ratios for Arg/Arg, Arg/Pro, Pro/Pro, and Arg/Pro or Pro/Pro in endometriosis versus controls was, 30.4% vs. 39.4%, 52.3% vs. 41.7%, 13.6% vs. 18.9%, and 65.9% vs. 60.6%, respectively, also showing no significant difference (67).

Wieser et al. (68) investigated the frequency of a 306-base pair insertion of the PV/HS-1 Alu subfamily in intron G of the progesterone receptor (*PR*) gene polymorphism PROGINS in women with and without endometriosis. The distribution of genotype frequencies was found to differ significantly in women with versus those without endometriosis. Women with endometriosis were found to have an increased frequency of the mutant allele (T2). The receptor changes caused by the PROGINS mutation may affect ligand and hormone binding and lead to insufficient regulation of estrogen receptor-driven proliferation. The authors stated that these results supported the theory of estrogen over-activation in endometriosis and suggested that PROGINS is associated with endometriosis in white women (68).

A group examined a common NOS polymorphism (a G→T substitution at nucleotide position 894, resulting in a change from Glu to Asp) and its potential association with endometriosis. The frequencies of the TG and TT genotypes were found to be significantly higher in women with endometriosis than in normal women, 87.2% versus 23%, respectively ($p < 0.001$). In addition, the frequency of the T allele was higher in endometriosis compared with the controls (63.8% versus 22.5%, $p < 0.001$). The authors found a 10-fold increased risk of developing endometriosis in patients possessing the mutant T allele (69).

The androgen receptor (*AR*) gene has a polymorphic cytosine, adenine, and guanine (CAG) microsatellite in exon 1 that codes for variable length glutamine repeats in the amino-terminal domain of the *AR* protein. In 2001, Hsieh et al. examined the

distribution of CAG repeats in 110 Chinese women with endometriosis versus 99 Chinese women without endometriosis. They found that women possessing one allele of genotype M (21 CAG repeats) had a statistically significant greater risk of developing endometriosis compared to women not possessing that genotype ($p = 0.007$). The primary genotype found in women without endometriosis was genotype L (20 repeats), although CAG repeats ranged from 9 repeats (genotype A) to 31 repeats (genotype W). The researchers believed that an AR gene polymorphism likely contributes to the pathogenesis of endometriosis, considering the AR protein is present in the endometrial tissue and pelvic organs, has been detected in endometriosis, adenomyosis, and endometrial carcinoma, and plays a role in modulating the cyclic change of the endometrium. The 21-CAG repeat polymorphism may be a variation of the AR gene that is involved in the formation of endometriosis (70).

A more recent study examined *AR* CAG polymorphisms in white Italian women. *AR* CAG length was analyzed in 105 women with endometriosis and 92 women without endometriosis (71). The number of repeats ranged from eight to 27 in the women with endometriosis and 11 to 27 in the control group, and therefore did not differ significantly ($p = 0.53$). Upon analyzing the endometriosis group's alleles based on stage of endometriosis, superficial and deep peritoneal implants, number of endometriomas, and adhesion scores, there were still no significant differences found based upon variables of severe disease. The difference in results between this study and that of Hsieh et al. may be due to differences in the *AR* gene locus between Asian and white populations. The researchers felt that *AR* CAG repeats might differently influence the development of endometriosis in different ethnic groups. Therefore, they believed more studies need to

be done involving different ethnic groups and utilizing specific endometriosis tissues versus blood (71).

The peroxisome proliferator-activated receptor (*PPAR*)-γ2 Pro-12-Ala polymorphism may be involved in the pathogenesis of endometriosis. DNA samples from 51 Caucasian women with endometriosis and 55 Caucasian women without endometriosis were analyzed for the Pro-12-Ala polymorphism in *PPAR-γ2*. The frequency of the Pro-12-Ala polymorphism in women with endometriosis was 0.41, which was significantly different from the controls (0.20, $p < 0.02$). However, differences between endometriotic stages were not statistically significant (stage I, 0.42; stage II, 0.43; stage III, 0.35; stage IV, 0.42; $p > 0.05$). *PPAR-γ2* is involved in transcriptional down-regulation of aromatase, an enzyme believed to be important in the pathophysiology of endometriosis. If the Pro-12-Ala polymorphism results in reduced down-regulation of aromatase, then higher levels of aromatase could stimulate implant growth by autocrine production of estrogen. Therefore, more studies are necessary to examine the roles of *PPAR-γ2* polymorphisms in endometriosis, and the effects *PPAR-γ2* agonists might have on the prevention and treatment of endometriosis. Thiazolidinediones (TZDs) are ligands of *PPAR-γ2*, and have been shown to inhibit monocyte migration. Therefore, treatment of endometriosis with TZDs could reduce cell proliferation and might also reduce the amount of inflammatory cells within the peritoneal cavity, which could assist in deterring the development and progression of endometriosis (72).

Although elevated levels of tumor necrosis factor alpha (*TNF-α*) have been found in the peritoneal fluid of women with endometriosis, three separate studies on *TNF-α* polymorphisms at positions 308 and 238 have not shown a statistical significance

between women with and without endometriosis. These studies were conducted on Taiwanese, Korean, and Caucasian women (73-75). *IL-6*, previously found to be increased in women with endometriosis (76, 77), was also examined for possible association of its G/C polymorphism at position 174 and the presence of endometriosis. In studies of both Korean and Caucasian women, no significant differences were found in between the *IL-6* genotypes (G/G, G/C, or C/C) and the presence of endometriosis (73, 78). There was, however, an association between the 174G allele and endometriosis among Caucasian women with chocolate cysts ($p = 0.037$). Serum *IL-6* levels were significantly higher in women with endometriosis ($p < 0.001$), but when broken down by genotype of the *IL-6* polymorphism, no significant differences were found (78).

Increased levels of interleukin-1 (*IL-1*) have been found within the peritoneal fluid and endometrium of women with endometriosis (79, 80). In addition, increased Interleukin-1β (*IL-1β*) mRNA expression has been found in peritoneal macrophages of women with endometriosis (81). An association has been shown between the *IL-1β* exon 5 polymorphism and chronic inflammatory and malignant diseases, as well as increased monocyte production of *IL-1β* (82, 83). Wieser et al. (84) investigated whether the incidence of the *IL-1β* exon 5 polymorphism was higher in women with endometriosis than women without endometriosis. They also examined whether the presence of this polymorphism is associated with altered *IL-1β* levels. Following pyrosequencing of DNA from 92 women with endometriosis and 69 controls, no significant difference was found between the groups regarding E1/E2 heterozygotes and E2/E2 homozygotes. In addition, no significant differences were found regarding the level of serum *IL-1β* and patient genotype (84).

Linkage Studies

There have been only a few attempts at linkage studies in families with endometriosis. One study examined 52 sister-pairs with Stage III-IV endometriosis utilizing three markers from the region of chromosome 1 (1p13) to which glutathione S-transferase M1 (*GSTM1*), a gene whose null mutation has been associated with endometriosis, had been mapped. The three markers were: D1S2635-10cM-D1S2844-5cM-D1S2762. No significant differences were found from expected values of identical-by-descent sharing. However, the researchers believed the ability to detect linkage may have been restricted due to recruitment of subjects from varied patient populations. This study also looked at the association of a null mutation of *GSTM1* with endometriosis and did not find a significant difference in the frequency of the mutation in women with endometriosis versus those without (85).

A recent study was the first to identify a major susceptibility locus for endometriosis by examining affected members from 1176 families. It found a region of chromosome 10q26 that exhibited significant linkage ($p = 0.047$), and another region that was considered to be suggestive of linkage on chromosome 20p13 with a maximum loss of odds (LOD) score of 2.09 (86).

Loss of Heterozygosity

Until 1997, few cytogenetic studies had been performed examining the incidence of somatic chromosomal changes in endometriotic tissues. The studies that had been done indicated no consistent chromosome alteration in patients with endometriosis, most likely due to mixed tissue specimens and techniques that did not enable direct detection of low levels of chromosomal aneuploidy. Shin et al. employed a multi-color fluorescence in-situ hybridization (FISH) approach to examine single cells and reported

non-random chromosome alterations, including trisomy 11 and monosomy 16 and 17 in late-stage disease (62). Their results suggested that chromosome-specific numerical abnormalities may be involved in the development and/or progression of endometriosis, possibly reflecting clonal expansion of chromosomally abnormal cells. Several oncogenes and tumor-suppressor genes had been mapped to chromosomes 11, 16, and 17, which also suggested that chromosomal loss or gain plays a role in the development and progression of endometriosis. They compared these findings to those of colorectal tumorigenesis, in which a series of somatic genetic changes involved the activation of oncogenes and loss of tumor-suppressor genes. They believed a similar process could be acting in endometriosis, in which the progression from mild to severe forms could be due to a series of somatic genetic changes that involve the activation of oncogenes and inactivation of tumor-suppressor genes (62).

Bischoff and Simpson (87) proposed that the pathogenesis of endometriosis could involve a multi-step process similar to that of neoplasia. Similar to cancer development, both mutations could be somatic or one could be a germ-line mutation and the second somatic. However, in contrast to the neoplasia model, not all of the genes in endometriosis pathogenesis would have to be oncogenes or tumor suppressor genes. Bischoff and Simpson believe that the first "hit" might involve a gene conferring an increased predisposition to adhesion and implantation of refluxed endometrial tissue (retrograde menstruation), including genes involved in the cytoskeleton, cell adhesion, or macrophage scavenging. The second gene, or "hit", could be a gene involved in supporting endometrial growth, such as genes involved in the ineffective metabolism of chemicals and/or toxins, causing build-up within the peritoneal cavity and oxidative

stress. They also hypothesize that additional hits could involve oncogenes and/or tumor suppressor genes, leading to uncontrolled cellular proliferation (88, 89).

LOH refers to a loss of DNA by deletion of an allele or an entire chromosome. Comparative genomic hybridization (CGH) is a method enabling location and mapping of genomic regions for chromosomal gains and/or losses. Regions showing an increased copy number may harbor dominant oncogenes, whereas regions with a decreased copy number may contain tumor suppressor genes. In a study of endometriotic lesions utilizing CGH, chromosomal imbalances occurred in 15 of 18 lesions examined. The DNA copy number abnormalities varied from 1 to 8 per lesion and were not related to the histological type or to the site of the endometriotic lesion. The most common regions of loss were located on 1p, involving at least 1p32-36 (50%), 5p (33%), 6q (27%), 7p14-p22ter (22%), and 22q12.3-qter (50%) segments. Less common regions of loss were 9q (22%), 16q (22%), and 17q (one case). Loss of heterozygosity involving chromosome 1 was common in all types and stages of endometriosis, including peritoneal implants, endometriomas, and umbilical nodules. Chromosomal gains were less common and localized to chromosomes 6q and 17q in two cases (90).

Kosugi et al. (91) also studied endometriosis utilizing FISH and observed a significant increase in the frequency of chromosome 17 aneuploidy in late-stage endometriosis as compared to normal endometrium. This increase in aneuploidy and heterogeneity among aneuploid cells demonstrated for the first time a possible role for somatic alterations in the chromosome 17 in the pathogenesis of endometriosis, although genetic alterations involving chromosome 17 had already been implicated in ovarian and breast cancer (91). Chromosome 17p13.1 contains the gene for tumor protein p53 (*TP53*), a transcription factor involved in cell death and cell-cycle regulation at G1.

Human epidermal growth factor receptor 2 (*HER-2/neu*) is located at 17q11.2-q2 and is an oncogene, transmembrane tyrosine kinase receptor, and member of the epidermal growth factor receptor (*EGFR*) family. 17q21 is the location of breast cancer 1, early onset (*BRCA1*), a tumor suppressor gene (87).

Specific Candidate Genes

Over 45 candidate genes have been associated with pathogenic hypotheses of endometriosis (http://www.well.ox.ac.uk/~krinaz/genepi_endo.htm). These include genes involved in retrograde menstruation and cervical stenosis (galactose-1-phosphate uridyl transferase), cell adhesion (E-cadherin; intercellular adhesion molecule-1), hormonal factors (catechol-O-methyltransferase; cytochrome P450, family 17; cytochrome P450, family 19; hydroxysteroid (17-beta) dehydrogenase 2; nuclear receptor subfamily 5, group A, member 1), hormone receptor variability (estrogen receptor 1; estrogen receptor 2; progesterone receptor), immunological factors (major histocompatibility complex, class I, A; major histocompatibility complex, class I, B; major histocompatibility complex, class I, C; major histocompatibility complex, class II, DR beta 1; major histocompatibility complex, class II, DP beta 1; major histocompatibility complex, class II, DQ beta 1; interleukin 1, alpha; interleukin 1, beta; interleukin 1 receptor antagonist; interleukin 4; interleukin 6; interleukin 10; luteinizing hormone, beta subunit; tumor necrosis factor-α), proteolytic enzymes (matrix metallopeptidases 1, 3, and 7), growth factors and tumor suppressor genes (interleukin 1 receptor, type 1; p21; p53; vascular endothelial growth factor), altered endometrial development (homeobox A10; homeobox A11), and detoxification (aryl hydrocarbon receptor; aryl hydrocarbon receptor repressor; aryl hydrocarbon receptor nuclear

translocator; cytochrome P450, family 1, subfamily A, polypeptides 1 and 2; cytochrome P450 family 1, subfamily B, polypeptide 1; glutathione S-transferase M1; glutathione S-transferase theta 1; N-acetyltransferases 1 and 2) (92).

A factor that could increase exposure to retrograde menstruation, and thus the risk of endometriosis, is cervical stenosis. Cervical stenosis could be caused by subtle Müllerian agenesis. Müllerian agenesis has been associated with impaired galactose metabolism, which can be caused by mutations in the galactose-1-phosphate uridyl transferase (*GALT*). The N314D and Q188R polymorphisms of *GALT* reduce its enzyme activity. Current evidence suggests a possible role for the N314D polymorphism in infertility, but not necessarily in endometriosis. One study found a significantly higher proportion of 33 infertile women with endometriosis (30%) carried at least one N314D allele compared to 111 healthy population controls (14%) (93). However, more recently, no difference was found in the N314D allele frequency between 85 affected women with a family history of endometriosis and 213 female and male population controls. In addition, no evidence of linkage to chromosome 9p was observed, where the *GALT* gene is located (94). Based on these results and other existing reports, it is not likely that the gene encoding the *GALT* protein, or a gene in its vicinity, is associated with endometriosis.

In 1993, a study reported that monkeys exposed daily to dioxin showed development of moderate to severe endometriosis in a dose-dependent manner (95). This prompted several studies on dioxin and endometriosis. It is conceivable that dioxin may cause endometriosis by transactivating the promoters of the cytochrome gene, giving rise to increased levels of cytochrome p-450 enzymes in endometrial tissues that reach pelvic peritoneal surfaces by retrograde menstruation. The enzymes may activate chemically

diverse procarcinogens in these tissues and give rise to development and growth of endometriotic implants (96).

The expression of *CYP1A1*, *CYP1A2*, *CYP1B1*, *AHR*, and *ARNT* was studied in human eutopic endometrial and endometriotic tissues. The transcript level of expression of *AHR*, *ARNT*, *CYP1A2*, and *CYP1B1* were the same in both endometriotic and eutopic endometrial tissues. *CYP1A1* transcript levels, however, were significantly higher in endometriotic tissues versus eutopic endometrium. *CYP1A1* genes catalyze the metabolic transformation of estrogen to catechol estrogens, which are short-lived estrogen metabolites that are speculated to play an important role in the growth and development of estrogen-responsive disorders such as breast cancer and endometriosis. *CYP1A1* overexpression in endometriosis may represent a common pathway for a direct dioxin effect and a dioxin-independent transactivation of the *CYP1A1* gene through aberrant expression of transcription factors in the tissue. Regardless of the role of dioxin, the end result of *CYP1A1* overexpression would be the development and growth of endometriotic tissue (97).

In the study of candidate genes that may play a role in the endometriosis pathogenesis, two that are of particular interest are the homeobox genes, *HOXA10* and *HOXA11*. *HOXA10* and *HOXA11* mediate embryonic development, including the development of the reproductive tract (98). They play an analogous role in endometrial development during the adult menstrual cycle, and their expression may possibly regulate growth and development of the human endometrium. *HOXA10* and *HOXA11* gene expression varies in response to sex steroids during the menstrual cycle, with dramatic up-regulation in the mid-secretory phase, the time of implantation. They are essential for implantation in the mouse, and have been shown to likely play a similar role in human

implantation. In a study of eutopic endometrium from women with and without endometriosis, the expected up-regulation of these two genes' expression was observed in the mid-luteal phase. However, in patients with endometriosis, an equivalent up-regulation of gene expression was not observed for either gene at the time of implantation. This failure to increase HOX gene mRNA levels did not depend on the stage of the disease and occurred despite in-phase endometrial histology. This failure of the normal increase in *HOXA10* and *HOXA11* mRNA levels to occur at the beginning of the window of implantation may be one mechanism responsible for endometriosis-related infertility (98).

In August, 2001, the OXEGENE and Australian (Genes Behind Endometriosis) studies, which both began in 1995, were merged into the International Endogene Study. The International Endogene Study represents a collaboration between the University of Oxford and the Australian Cooperative Research Centre for Discovery of Genes for Common Human Diseases (Gene CRC). As of April 2002, the combined data set consisted of 2,932 families. This is the largest clinical resource for linkage and association studies in endometriosis ever assembled. This merger increases the power to detect genes predisposing women to endometriosis and it demonstrates the value of large-scale international collaboration. By the end of 2002, genotyping was completed on the affected sibling-pair (ASP) families and linkage associations were better characterized, utilizing a genome-wide scanning of informative polymorphic microsatellite markers to identify regions of significant excess sharing in affected siblings for given genetic markers that are identical by descent (IBD) from their parents. This information will lead to candidate gene testing in those regions associated with endometriosis (99).

Recently, Zondervan, Cardon, and Kennedy, announced the development of a web site which incorporates evidence of genetic variants associated with endometriosis and new etiological hypotheses, as well as links to up-to-date genomic information relevant to the candidate genes from a range of bioinformatics databases. The web site, http://www.well.ox.ac.uk/~krinaz/genepi_endo.htm, contains a regularly updated review of opinions on etiological hypotheses and supporting biological, clinical, and epidemiological evidence, tables summarizing candidate genes investigated in association studies, hyperlinks to information about each gene's location, structure, function (if known), and maps of neighboring polymorphic markers held in Internet-based bioinformatics databases, including Online Mendelian Inheritance in Man (OMIM), Genecards, National Center for Biotechnology Information (NCBI) Locuslink, Ensembl, the University of California Santa Cruz (UCSC) Human Genome Browser or Golden Path, and the Single Nucleotide Polymorphism database (dbSNP). It also contains literature references with hyperlinks to abstracts published in PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) for each candidate gene. The team is currently conducting bimonthly searches of PubMed to find new association studies that present findings for or against association between endometriosis and specific genetic variants. They believe that this website will be of great benefit and will be a useful information tool for endometriosis researchers (92).

As a complex and multifactorial disorder, endometriosis is ideally suited as a target for genome-wide scanning. Techniques such as subtractive cDNA hybridization have identified glycodelin-A as a specific product of human endometrium believed to play an important role in embryonic receptivity (100). Microarray studies have shown that mRNA expression of glycodelin-A was upregulated in normal mid-secretory

endometrium, but reduced in mid-secretory eutopic endometrium of women with endometriosis. Other techniques, including differential display polymerase chain reaction (PCR) and comparative genomic hybridization have assisted in identifying up-regulation of proteins and chromosome regions that are altered in endometriosis. The field of bioinformatics is evolving rapidly, and genomic and proteomic technologies are poised to revolutionize the diagnosis and treatment of endometriosis and other diseases (100).

Microarray Studies involving Endometriosis

Eutopic versus Ectopic Microarray Studies

Genomic studies of endometriosis utilizing microarrays were first presented and published only a few years ago. Eyster et al. (5) were the first to publish a paper on utilizing DNA microarray analysis and endometriosis. This pilot study consisted of recruiting three women scheduled for surgery for endometriosis. The women were not on hormonal therapy and were in the follicular phase of their menstrual cycle when the surgery was performed. Eutopic endometrial and ectopic endometriomal samples were obtained. Total ribonucleic acid (RNA) extracted from these tissues was hybridized to Research Genetics Named Human Genes GeneFilter GF211 microarrays, which contain 4,133 human genes. 1,028 of these genes were expressed in the eutopic and/or ectopic endometrium. Eight genes displayed differential gene expression; this expression was greater in ectopic endometrium in all eight instances (5).

The eight genes showing greater expression in ectopic endometrium were β-actin, α-2 actin, vimentin, 40S ribosomal protein S23, Ig-λ light chain, Ig germline H chain G-E-A region γ-2 constant region, complement component 1 S subcomponent, and major

histocompatibility complex class 1,C. Following comparisons of the 1,028 genes expressed in endometrium with sets of genes expressed in other tissues, only 75 genes were unique to the endometrium. Therefore, of the eight genes differentially expressed between eutopic and ectopic endometrium, six were unique to the endometrium (vimentin, 40S ribosomal protein S23, Ig-λ light chain, Ig germline H chain G-E-A region γ-2 constant region, complement component 1 S subcomponent, and major histocompatibility complex class 1,C), while β-actin and α-2 actin have been found active in other tissues as well. In fact, β-actin is often used as a housekeeping gene for the normalization of expression of other genes (5).

In addressing the differential gene expression of the other six genes, the authors first looked at the invasive properties of endometriotic tissues. Vimentin is an intermediate filament involved in cytoskeletal integrity, therefore, it was believed that the invasive nature of endometrial implants might explain the up-regulation of vimentin, as well as β-actin and α-2 actin. The authors also cited reports of the effects of estrogen on the synthesis and expression of vimentin (via the increased demand for lipid droplets in steroidogenic cells and the necessary movement of these droplets into mitochondria via rearrangements of cytoskeletal elements), β-actin, and 40S ribosomal protein 23. In all three cases, they speculated that increased synthesis and/or presence of estrogen could result in increased expression of these genes in endometriotic implants (5).

The increased expression of Ig-λ light chain, Ig germline H chain G-E-A region γ-2 constant region, complement component 1 S subcomponent, and major histocompatibility complex class 1,C was considered to strengthen the importance of immune system dysfunction in endometriosis. The authors stated that B cells were known to be present in endometrial implants, although a difference between eutopic and

ectopic endometrium was not known. In a normal immune response, they would have expected an increase in the expression of the Ig-λ light chain as well as the κ light-chain gene, but this gene was not present on the GeneFilter microarray they utilized (5).

The differential gene expression they were most interested in was that of the Ig germline H chain G-E-A region. Normally, this gene undergoes rearrangements, selective transcription, and RNA processing that results in the synthesis of Ig heavy chains. According to the authors, the expression of this gene in its entirety suggested aberrant gene expression, because no cell type should express this gene in its unmodified germline state (5). Therefore, they considered this gene to be appealing for further study and a potential diagnostic tool for endometriosis. Their study also showed that microarray analysis could be utilized to study differential expression in genes in eutopic and ectopic endometrium (5).

At the 58th Annual ASRM meeting, a pilot study was presented that compared eutopic endometrial gene expression with that of chocolate cyst ectopic endometrium. Eutopic endometrial tissue was obtained from three women with endometriosis, as well as ectopic endometrial tissues from chocolate cysts. A human cDNA microarray system of 9,600 genes, including known regulatory genes and expressed sequence tags were utilized to study differential gene expression. 163 genes were found to be up-regulated three-fold or higher in ectopic endometrium, while only 14 genes were up-regulated in eutopic endometrium. Six genes were studied further by immunohistochemistry. These included B-cell leukemia/lymphoma 2 (*Bcl-2*), cyclin B1, vascular cell adhesion molecule 1 (*VCAM-1*), bone morphogenetic protein 4 (*BMP-4*), transforming growth factor beta (*TGF-β*) receptor, glutathione S-transferase A2 (*GSTA2*), and cell differentiation antigen 36 (*CD36*). Immunostaining results validated the microarray

studies, as all six genes were over-expressed in ectopic endometrium at the protein level (101).

A more comprehensive investigation of the genes involved in the pathogenesis of endometriosis was conducted utilizing cDNA microarrays consisting of 23,040 genes to study differential gene expression of ovarian endometrial cysts. Endometrial cysts were obtained from 23 women undergoing cystectomy. Nine participants were in the proliferative phase of the menstrual cycle, and 14 were in secretory phase at the time of surgery (102). Eutopic endometrial tissues were obtained as controls from seven of the proliferative phase participants and seven secretory phase participants. Epithelial cells were isolated from all samples, followed by RNA extraction (102).

Three rounds of RNA amplification were performed on the cyst epithelial cells, and two rounds performed on the control samples. Two universal controls were prepared, one from the eutopic amplified RNA (aRNA) mixture from the seven patients in proliferative phase, and the other from the seven secretory phase patients' aRNA. One set of expression comparisons was made between cysts in the nine proliferative phase patients versus the universal control of eutopic endometrium from the seven women in that phase. The other set of comparisons was made between the 14 cysts collected from women in the secretory phase versus the universal control prepared from the seven women in that phase (102).

Fifteen genes, including two expressed sequence tags (ESTs), were up-regulated in 70% of the 23 cases. Forty-two genes, including 15 ESTs, were up-regulated only in the proliferative phase, and 40 genes, including 10 ESTs, were up-regulated only in the secretory phase. Some genes were up-regulated exclusively in one phase or the other: S100 calcium-binding protein A13 (*S100A13*), myosin regulatory light chain 2, and

smooth muscle isoform (*MYRL2*) were up-regulated only in the proliferative phase; testis specific-like protein, Y-encoded (*TSPYL*) and four and a half LIM domains 2 (*FHL2*) were up-regulated only in the secretory phase (102).

The study also demonstrated down-regulation of numerous genes in the endometriotic cysts. Three hundred thirty-seven genes, including 164 ESTs, were commonly under-expressed in greater than 70% of the cysts collected from women both in proliferative and secretory phase. One hundred forty-four genes, including 41 ESTs, were under-expressed only in the proliferative phase, and 835 genes, including 438 ESTs, were down-regulated only in the secretory phase (102). Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was utilized to confirm the differences in expression indicated by the microarray studies (102).

Several genes involved in the immune system were found to up-regulated throughout the menstrual cycle, including *HLA-DPA1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DRA*, *HLA-DRB1*, and *CD14*. Genes encoding complements factors C3, BF, C1S, C1R, C2, CEBPD, and HLA-F were up-regulated primarily during the secretory phase. Up-regulation of genes involved in the immune system was hypothesized to be involved in poor reproductive outcomes, while increased expression of HLA antigens could involve recognition and presentation by macrophages, leading to an autoimmune reaction via endometrial autoantibodies. The increased expression of complement components were inferred to result from severe inflammation, due to the presence of endometriotic lesions, which could potentially damage fallopian tubes and ovaries, leading to infertility. Transforming growth factor, beta 1 was also found to be up-regulated throughout the menstrual cycle. Its role in inhibiting natural killer cell activity, while inducing angiogenesis and proliferation of endometrial stromal cells, was suggested to potentially

play a part in the progression of endometriosis and infertility. Other genes with increased expression included ribosomal proteins S23, L11, and S11, whose synthesis is know to increase in response to estrogens (102).

Genes involved in embryo protection, tumor suppression, and apoptosis were found to be down-regulated throughout both the proliferative and secretory phases of the menstrual cycle. Oviductal glycoprotein 1 (*OVPG1*), which is involved in protecting the early embryo and fallopian tube from potentially noxious extracellular environments, was found to be down-regulated throughout the entire menstrual cycle (102). Decreased expression of this gene was speculated, therefore, to lead to infertility in women with endometriosis. Down-regulation of *TP53* and tumor protein p53 binding protein 2 (*TP53BP2*) were believed to support the notion that endometriosis, although a benign condition itself, exhibits tumor-like features, including cellular proliferation, invasion, and neoangiogenesis. In addition, the expression of growth arrest and DNA-damage-inducible 34 (*GADD34*), 45 alpha (*GADD45A*), and beta (*GADD45B*) proteins, as well as tumor protein p53 inducible protein 11 (*PIG11*), was found to be down-regulated throughout the menstrual cycle. The researchers felt that decreased expression of these genes in the endometrial epithelial cells might affect apoptotic signaling and enhance the tumor-like characteristics of endometriosis (102).

Gene expression has also been compared between different types of endometriosis utilizing microarray technology. In a study by Mahadevappa et al. (103), gene expression was compared between normal endometrium, peritoneal endometriosis, and ovarian endometriosis. Samples included eighteen of normal endometrium, twelve peritoneal lesions, and fifteen ovarian endometriomas. cDNA was hybridized to Human U133A oligonucleotide arrays (Affymetrix, Inc.) containing approximately 22,000 genes.

Candidate genes were identified by both parametric (Students t-test) and non-parametric methods (Mann-Whitney test), segregated by hierarchical clustering, and a select group confirmed by quantitative reverse transcription-polymerase chain reaction (QRT-PCR). Candidate genes identified and confirmed included steroidogenic acute regulatory protein (*STAR*), myosin, heavy polypeptide 11 (*MYH11*), dienelactone hydrolase (*DLH*), and wingless-type mouse mammary tumor virus (*MMTV*) integration site family member 1 (*WNT1*) inducible signaling pathway protein 2 (*WISP2*) (103).

Transducer of ErbB (Tob)-1

IL-1β, a product of peritoneal macrophages, performs several important roles in the establishment and maintenance of endometriotic lesions (104). It has been shown to stimulate angiogenesis and monocyte chemotaxis in endometriotic cells, as well as induce mitogenesis in other types of cells. Lebovic et al. (105) explored the relationship between *IL-1β* and the survival of endometriotic tissue. They wanted to find a mechanism through which the exaggerated immune response seen with endometriosis might be linked to the enhanced survival of endometriotic lesions through *IL-1β* induction of cell-cycle regulatory proteins. Therefore, they studied the effect exposure to *IL-1β* had on gene expression of normal endometrial stromal cells versus endometriotic stromal cells (105).

Four eutopic endometrial samples were collected from women undergoing laparoscopy or laparotomy, with pathologic diagnoses of uterine leiomyomata, but no clinical evidence of endometriosis, and ovarian endometrioma biopsies were collected from four other women. Normal endometrial stromal and endometriotic stromal cells were separated from glandular epithelial cells and cultured with *IL-1β*. Atlas Human

cDNA Expression arrays (Clontech), with 597 genes, were utilized to screen for affected mRNA expression due to *IL-1β* exposure (105).

Only one gene, transducer of ErbB (*Tob*)-1, showed down-regulated expression due to exposure to *IL-1β*. Although *Tob-1* expression was reduced by 48% in endometriotic stromal cells following *IL-1β* treatment, in normal endometrial stromal cells, the effect was significantly less (16% inhibition). The inhibitory effect of *IL-1β* on *Tob-1* was studied further by Northern blot hybridization. Following 12-hour incubations with *IL-1β*, the treated endometriotic stromal cells showed a 25 ± 5% decrease in *Tob-1* mRNA induction ($p < 0.05$) versus untreated endometriotic stromal cells. In the normal endometrial stromal cells, however, a < 3% in *Tob-1* mRNA decrease was observed in the treated versus untreated cells (105).

Tob-1 is a tumor suppressor gene that inhibits cell growth by sequestering cells in G_0/G_1 phases of the cell cycle (105). The gene for *Tob-1* is located on 17q21, and functional loss of this chromosome has been implicated in endometriotic lesions (91). However, *Tob-1* has not been sufficiently studied in other cell systems; therefore the impact of a 25% reduction in mRNA expression is not known. Nonetheless, the researchers acknowledged that the suppression of *Tob-1* in endometriotic stromal cells by *IL-1β* provided a mechanism for the enhanced growth potential of endometriotic lesions (105).

Eutopic Endometrial Microarray Studies

A few studies have examined eutopic endometrium in women with and without endometriosis. The first was presented at the VIII World on Endometriosis. Chen et al. obtained endometrial biopsy samples from four women and mRNA extracted from the

tissues and hybridized to human cDNA microarrays with 9,600 genes, including known regulatory genes and expressed sequence tags. Seventeen genes had higher expression in endometrium from the normal women during the proliferative phase, and 197 genes showed greater expression in the eutopic endometrium of women with endometriosis (6). The genes differentially expressed included cell growth-related factors (e.g., epidermal growth factor, insulin-like growth factor-II), cell adhesion molecules (e.g., thymus cell surface antigen 1, integrin beta-4 binding protein), signal transduction molecules (e.g., ras-related oncoprotein), apoptosis-related factors (e.g., death-domain related genes), hormones/cytokines (e.g., epidermal growth factor, *TGF-β*, neuropeptide Y receptor, natural killer cells-stimulated proliferative factor), cytoskeleton/extracellular matrix proteins (e.g. keratin 18), and some ESTs (6).

A second study comparing eutopic endometrium in women with versus women without endometriosis was presented as a poster at the 59th Annual meeting of the ASRM. Endometrial biopsies were obtained from three women with endometriosis and three age and cycle-matched controls without endometriosis. The samples were collected during the luteal phase of the menstrual cycle (days 25-27). Total RNA from each sample was hybridized to a human Genechip containing 33,000 transcripts (Affymetrix, Inc.). Three genes, cystatin B (stefin B), heat shock 70kD protein 1B, and enolase (1, alpha), were identified whose expression was significantly higher in the endometrium of the women with endometriosis. The expression of insulin-like growth factor binding protein 5, tyrosine 3-monooxygenase, tryptophan 5-monooxygenase activation protein, and epsilon polypeptide (14-3-3 protein) was significantly down-regulated in women with endometriosis versus normal women (106).

One of the most comprehensive studies of endometrial gene expression in women with versus those without endometriosis was conducted by Kao et al (10). Initial results were presented at the VIII World Congress on Endometriosis (107) and at the National Institutes of Health workshop "Endometriosis: Emerging Research and Intervention Strategies" (108). Eutopic endometrial samples were collected from eight women with and twelve women without surgically-confirmed endometriosis. Collection of samples was timed to the luteinizing hormone (LH) surge, therefore tissue biopsies were performed during the window of implantation, eight to ten days following the LH surge. RNA was hybridized to Affymetrix Genechip Hu95A oligonucleotide microarrays composed of 12,686 genes. Genes of different expression fold changes were randomly selected for validation by RT-PCR and/or Northern or dot-blot analyses (10).

During the window of implantation, 91 genes, including 28 ESTs, were up-regulated in the endometrium of women with endometriosis versus those without, and 115 genes, including 29 ESTs, were down-regulated in women with endometriosis versus without. Therefore, 63 genes were up-regulated by a minimum of two-fold and 86 genes were down-regulated by two-fold or greater in the eutopic endometrium of women with endometriosis as compared to expression in normal women. Genes with functions involving or categorized as apoptosis, protein or RNA processing, transporter proteins, zinc metalloenzymes, DNA repair, immune function, secretory proteins, signal transduction, cell surface proteins, and transcription factors, as well as other unspecified pathways, were up-regulated according to the study's algorithm to the maximum of 100-fold. The genes demonstrating the greatest down-regulation (reaching a maximum of 100-fold) were involved in calcium binding, RNA polymerase, serine protease/inhibition,

signal transduction, transcription factors, immune function, cell surface proteins, cytokine receptor genes, ion transporters, and secretory proteins (10).

Kao et al. then compared the results of this study with that of a previous set of microarray experiments they had performed comparing gene expression in normal human endometrium during the implantation window with expression in the late proliferative phase (109). Three groups of target genes were identified, based on expression within the window of implantation and presence of endometriotic disease. Group 1 consisted of eight genes normally up-regulated in the window of implantation that were significantly down-regulated in women with endometriosis (10). These included interleukin-15 (*IL-15*), proline-rich protein (*PRP*/*C4BP*), immediate early response protein B61 (ephrin-A1), Dickkopf-1 (*DKK1*), glycodelin, *N*-acetylglucosamine-6-*O*-sulfotransferase (*GlcNAc6ST*), putative lymphocyte G0/G1 switch gene (*G0S2*), and purine nucleoside phosphorylase. Group 2 consists of semaphorin E, neuronal olfactomedin-related endoplasmic reticulum localized protein mRNA, and Sam68-like phosphotyrosine protein α (*SALP*), which are genes normally down-regulated during the window of implantation, but were up-regulated in women with endometriosis. The final group (Group 3) was composed of one gene, neuronal pentraxin II, which is normally down-regulated during the window of implantation and displayed even greater down-regulation in women with endometriosis (10).

The genes within these three groups, as well as a few others, were hypothesized to play roles in implantation failure and infertility, as well as in the pathogenesis of the disease. This was due to the differences in gene expression during the window of implantation in women with versus without endometriosis. The genes considered as candidates for implantation failure and infertility in endometriosis included *GlcNAc6ST*,

olfactomedin, *C4BP*, *IL-15*, *DKK1*, purine nucleoside phosphorylase, neuronal pentraxin II, glycodelin, S100 calcium binding protein A3 (*S100E*), and bile salt export pump (*BSEP*). The genes proposed to contribute to the pathogenesis of endometriosis included integrin α, aromatase, progesterone receptor (*PR*), ephrin-A1, *G0S2*, kallikrein, semaphorin E, and *SALP* (10).

Tissue Microarrays

Most recently, the first experiment utilizing tissue microarray technology in studying endometriosis was performed to assess the differential gene expression of estrogen receptor (*ER*)-α, *PR*, matrix metalloproteinases (*MMP*s)-1, 7, and 11, dosage-sensitive sex adrenal hypoplasia congenital critical region on the X chromosome gene 1 (*DAX-1*), and caveolin-1 (*CAV-1*). Tissue samples were collected from fifty-one women with endometriosis and nine controls. Of the women with endometriosis, 10% had mild endometriosis, 23% moderate, and 67% had severe endometriosis. Samples collected included eutopic endometrium (25%), ovarian endometriosis (48%), and peritoneal endometriosis (27%). Core biopsies of two millimeters were assembled onto tissue microarrays, and immunohistochemistry was used to assess the expression of the aforementioned genes. The extent of antibody expression (negative, weakly positive, or strongly positive) was correlated to the presence, severity, and site of endometriosis (110).

The expression of *ER-α* was significantly greater in the endometriotic tissues versus the controls ($p = 0.009$), and eutopic endometrium from women with endometriosis showed significantly stronger *ER-α* than controls ($p < 0.0001$). Endometriotic tissues also demonstrated significantly down-regulated expression of *PR*

as compared to controls ($p = 0.003$). Odds for disease were significantly lower with strong expression of *PR*, compared with those with negative and mild expression (OR 0.06). In addition, *MMP-1* showed a trend of being over-expressed, especially in the peritoneal implants ($p = 0.05$). The expressions of *MMP-7*, *MMP-11*, *DAX-1*, and *CAV-1* did not display any significant differences between endometriotic and control tissues. The researchers believed that increased expression of *ER-α* and decreased expression of *PR* in endometriotic tissues indicated the presence of altered receptivity of endometriotic implants (110).

CHAPTER III

MATERIALS AND METHODS

Research Subjects

The population of interest was women of reproductive age, both with and without endometriosis. The study sample was women of reproductive age, at least eighteen years of age, who were scheduled for laparoscopic surgery. Normal subjects were recruited from women undergoing laparoscopic surgery for tubal ligation, while endometriosis subjects were recruited from women undergoing laparoscopic surgery for evaluation of pelvic pain and/or infertility believed to be associated with endometriosis. Each potential study participant was read an informed consent letter approved by the Oklahoma State University Center for Health Sciences College of Osteopathic Medicine Institutional Review Board (Appendix II). At this time, the potential participants' questions were answered and all procedures explained.

Once enrolled, each study participant was assigned a number. The list identifying patient name and number was kept in a classified folder to which only the primary researcher or physicians associated with the study had access. All participants were asked to fill out a questionnaire on their medical history, family history of disease, infertility, surgical history, and prescribed and herbal medications use, as well as rate their pain, if applicable, on a gynecological pain scale. At the conclusion of the study, the list identifying patient name and number were destroyed.

Sample Collection

An obstetrician/gynecologist performed the laparoscopic surgeries at Tulsa Regional Medical Center. For the normal participants enrolled in the study, three samples were taken: (1) an endometrial biopsy, (2) peritoneal fluid, and (3) normal pelvic peritoneum. For the endometriosis participants, four samples were collected: (1) peritoneal fluid, (2) normal pelvic peritoneum, (3) endometriotic lesion, and (4) an endometrial biopsy. All samples were collected utilizing sterile technique while the participant was under general anesthesia.

Normal Participants

Endometrial Biopsy. The endometrial biopsy was obtained prior to commencement of the laparoscopic procedure. The endometrial biopsy was collected through insertion of an Endocurette® Endometrial Suction Curette (Utah Medical Products, Inc., Catalog # CUR-100) through the cervix into the uterus. The suction plunger was pulled out and the curette pushed into the uterus and pulled out several times while rotating the instrument, in order to collect eutopic endometrial cells. The curette was then placed into a 15ml centrifuge tube filled with 10 ml RNA*later*™ (Ambion, Catalog #7020) and placed on ice. Per RNA*later*™ recommendations, the sample was stored at 4ºC overnight, and then transferred to -20ºC and stored until RNA extraction.

Normal Pelvic Peritoneum. A biopsy of normal pelvic peritoneum was obtained utilizing a grasping laparoscopic forcep instrument. The sample was put into 5 ml of

RNA*later*™ and placed on ice. The sample was stored at 4ºC overnight, and then transferred to -20ºC and stored until RNA extraction.

Endometriosis Participants

For endometriosis participants, no samples were collected unless endometriosis was visually confirmed. If endometriosis was present, the operating physician visually staged the endometriotic lesions, based upon the revised ASRM classification of endometriosis (16). The endometrial biopsy, peritoneal fluid, and normal pelvic peritoneal biopsy were obtained in the same manner as in the normal participants.

Endometriosis. A biopsy of endometriotic tissue was collected utilizing a grasping laparoscopic forcep instrument. The biopsy was collected from the uterine cul-de-sac and placed into 5 ml RNA*later*™. The sample was stored at 4ºC overnight, and then transferred to -20ºC for storage.

Total RNA Extraction

Endometrial Biopsies

RNA was extracted from endometrial samples utilizing the RNeasy Mini Kit (Qiagen, catalog # 74104). Endometrial tissue was removed from each 15 ml centrifuge tube using sterile forceps and weighed. The tissue was then placed in 600 µl Buffer RLT with β-Mercaptoethanol (β-ME) in a 1.5-ml microtube. The Buffer RLT/ β-ME mixture was freshly made prior to beginning the RNA extraction protocol; 10 µl β-ME was added to 1 ml Buffer RLT. The tissue was then homogenized using a rotor-stator homogenizer until the sample was uniformly homogenous (20-40 seconds). The lysate was then

further homogenized by passing ten times through a 20-gauge needle fitted to a sterile syringe.

The lysate was centrifuged at maximum speed (13,000 rpm) for three minutes in a microcentrifuge. The supernatant was transferred to a new microtube by pipetting, and 600 µl of 70% ethanol was added to the lysate and mixed immediately by pipetting. One-half of the sample (600 µl) was applied to an RNeasy mini column placed in a 2 ml collection tube. The tube was closed gently and microcentrifuged for 15 seconds at 10,000 rpm. The flow-through was discarded, and the remaining 600 µl was applied to the column. The tube was closed gently and microfuged at 10,000 rpm for 15 seconds, after which the flow-through was discarded. Buffer RW1 (350 µl) was pipetted onto the RNeasy mini column, followed by a 15-second microcentrifugation at 10,000 rpm to wash. Afterwards, the flow-through was discarded.

The DNase I incubation mix was prepared by added 10 µl DNase I stock solution to 70 µl Buffer RDD. The DNase I stock solution and Buffer RDD were mixed gently by inverting the tube. The DNase I incubation mix was pipetted directly onto the RNeasy silica-gel membrane and then incubated at room temperature for 15 minutes. Following the incubation, 350 µl Buffer RW1 was pipetted onto the RNeasy mini column, followed by a 15-second microcentrifugation at 10,000 rpm. The RNeasy mini column was then transferred to a new 2 ml collection tube.

500 µl Buffer RPE was pipetted onto the RNeasy column. The tube was closed gently and microcentrifuged for 15 seconds at 10,000 rpm to wash the column. The flow-through was discarded, and another 500 µl Buffer RPE was pipetted onto the RNeasy column. The tube was closed gently and microcentrifuged for two minutes at 10,000 rpm to dry the RNeasy silica-gel membrane. The RNeasy column was placed in a

new 1.5 ml microtube and microcentrifuged at full speed for one minute to eliminate any chance of possible Buffer RPE carryover.

To elute, the RNeasy column was transferred to a new 1.5 ml microtube. 30 µl RNase-free water was pipetted directly onto the RNeasy silica-gel membrane. The tube was closed gently and incubated at room temperature for ten minutes. Following the incubation, the tube was microcentrifuged for one minute at 10,000 rpm to elute. The eluate was reapplied to the column for a second elution. The tube was incubated for another ten minutes at room temperature and then microcentrifuged for one minute at 10,000 rpm to elute. Two microliters of the eluate were placed in a separate 0.5 ml microtube for subsequent RNA concentration evaluation. Both the 1.5 ml and 0.5 ml with total RNA were stored at -80ºC.

Normal Pelvic Peritoneum

RNA was extracted from normal pelvic peritoneum utilizing the RNeasy Micro Kit (Qiagen, catalog # 74004). Normal pelvic peritoneum was removed from each 15 ml centrifuge tube using sterile forceps and weighed. The tissue was then placed in 350 µl Buffer RLT with β-ME in a 1.5-ml microtube. (The Buffer RLT/ β-ME mixture was made prior to beginning the RNA extraction protocol. 10 µl β-ME was added to 1 ml Buffer RLT.) The tissue was then homogenized using a rotor-stator homogenizer until the sample was uniformly homogenous (20-40 seconds). The lysate was then further homogenized by passing ten times through a 20-gauge needle fitted to a sterile syringe.

The lysate was then spun at maximum speed (13,000 rpm) for three minutes in a microcentrifuge. The supernatant was transferred to a new microtube by pipetting. 350 µl of 70% ethanol was added to the lysate and mixed immediately by pipetting. The

sample was applied to an RNeasy MinElute Spin Column placed in a 2 ml collection tube. The tube was closed gently and microcentrifuged for 15 seconds at 10,000 rpm. The flow-through was discarded. 350 µl Buffer RW1 was pipetted into the RNeasy MinElute Spin Column, followed by a 15-second microcentrifugation at 10,000 rpm to wash the column. The flow-through was discarded.

The DNase I incubation mix was prepared by added 10 µl DNase I stock solution to 70 µl Buffer RDD. The DNase I stock solution and Buffer RDD were mixed gently by inverting the tube. The DNase I incubation mix was pipetted directly onto the RNeasy MinElute silica-gel membrane and then incubated at room temperature for 15 minutes. Following the incubation, 350 µl Buffer RW1 was pipetted onto the RNeasy MinElute Spin Column, followed by a 15-second microcentrifugation at 10,000 rpm. The RNeasy MinElute Spin Column was then transferred to a new 2 ml collection tube.

500 µl Buffer RPE was pipetted onto the RNeasy MinElute Spin Column. The tube was closed gently and microcentrifuged for 15 seconds at 10,000 rpm to wash the column. The flow-through was discarded. 500 µl 80% ethanol was pipetted onto the RNeasy MinElute Spin Column. The tube was closed gently and microcentrifuged for two minutes at 10,000 rpm to dry the RNeasy silica-gel membrane. The RNeasy column was placed in a new 1.5 ml microtube. The cap of the spin column was opened and microcentrifuged at full speed for one minute.

To elute, the RNeasy MinElute Spin Column was transferred to a new 1.5 ml microtube. 14 µl RNase-free water was pipetted directly onto the RNeasy MinElute silica-gel membrane. The tube was closed gently and incubated at room temperature for ten minutes. Following the incubation, the tube was microcentrifuged for one minute at 10,000 rpm to elute. The eluate was reapplied to the column for a second elution. The

tube was incubated for another ten minutes at room temperature and then microcentrifuged for one minute at 10,000 rpm to elute. Two microliters of the eluate were placed in a separate 0.5 ml microtube for subsequent RNA concentration evaluation. Both the 1.5 ml and 0.5 ml microtubes with total RNA were stored at -80ºC.

RNA Concentration

Spectroscopy

RNA concentration was determined utilizing a NanoDrop® ND-1000 Spectrophotometer. With the sampling arm open, a 1.5 µl sample of total RNA was pipetted onto the lower measurement pedestal. The sampling arm was closed and a spectral measurement initiated using the operating software on the PC. The sample column was automatically drawn between the upper and lower measurement pedestals and the spectral measurement made. When the measurement was completed, the sampling arm was opened the sample was wiped from both the upper and lower pedestals using a Kim-Wipe. The pedestals were then cleaned with de-ionized water after each last measurement.

RNA Amplification

First Strand cDNA Synthesis with dT Primer

For each RNA sample, an RNA/primer mix was prepared in a microtube on ice. This was composed of 1-9 µl total RNA, not exceeding 2 µg, 2 µl SenseAmp dT24 RT primer (50 ng/µl), and nuclease free water to a volume of 11 µl. This was heated to 80ºC

for 10 minutes, and then placed on ice immediately for 2 minutes. After a brief microfuge, the RNA/primer mix was returned to ice.

A Master Mix (MM1) was prepared for each reaction in a separate tube on ice. MM1 was comprised of 4 µl 5X First Strand Buffer, 2 µl 0.1M dithiotreitol (DTT), 1 µl Superase-In, 1 µl dNTP Mix, and 1 µl Superscript II, for a total volume of 9 µl. MM1 and the RNA/primer mix were combined, mixed gently, microfuged, and then incubated at 42ºC for 2 hours. Following the incubation, the cDNA reaction was microfuged briefly. 80 µl 1X TE Buffer was added for a final volume of 100 µl.

Purification of cDNA

The cDNA was purified using the MinElute PCR Purification Kit (Qiagen, catalog # 28006) as follows. 500 µl Buffer PB was added to the 100 µl cDNA sample and mixed. The cDNA mixture was applied to the MinElute column and centrifuged for 1 minute at 10-14,000 x g (~13,000 rpm) in a conventional tabletop microcentrifuge. The flow-through was discarded and the MinElute column placed into the same collection tube. 750 µl Buffer PE was added to the MinElute column and centrifuged for 1 minute. The flow-through was discarded and the MinElute column placed into the same collection tube. 500 µl 80% ethanol was added to the MinElute column and centrifuged for 2 minutes. The flow-through was discarded and the MinElute column placed into the same collection tube. The column cap was opened and placed in the microfuge, with the cap opposite the direction of rotation of the rotor to avoid breaking the cap off, and centrifuged for 5 minutes. The MinElute column was placed into a clean, labeled 1.5 ml microfuge tube. To elute cDNA, 10 µl Buffer EB was added to the center of the column membrane, and the tube incubated at room temperature for 2 minutes, followed by a two-

minute centrifugation. The column was discarded. If the eluted cDNA was less than 10 µl, the volume was brought to 10 µl with nuclease free water.

Tailing of First Strand cDNA

The purified cDNA was heated to 80ºC for 10 minutes. It was then placed on ice immediately for 2 minutes. The cDNA was briefly microfuged and then returned to ice. For each reaction, a Master Mix (MM2) was prepared in a separate tube on ice. MM2 was composed of 2 µl 10X Reaction Buffer, 2 µl Nuclease Free Water, 4 µl 10mM dTTP, and 2 µl TdT Enzyme. MM2 was combined with the cDNA for a total volume of 20 µl, mixed gently, microfuged, and incubate in a 37ºC heat block for 3 minutes, paying attention, so as to not exceed 3 minutes. The reaction was stopped by heating to 80ºC for 10 minutes, followed by a brief microfuge, and then cooling to room temperature for 1-2 minutes.

T7 Promoter Synthesis

2 µl T7 Template Oligo was added to 6 ul of nuclease free water, briefly vortexed, and microfuged. 2 µl of this dilution was added to the tailed cDNA, for a final volume of 22 µl, which was then vortexed briefly and microfuged. It was then incubated at 37ºC for 10 minutes to anneal the strands. To each reaction, 1 µl 10X Reaction Buffer, 1 µl dNTP mix, and 1 µl Klenow Enzyme was added. This mixture was mixed gently and microfuged, followed by a 30-minute incubation at room temperature. The reaction was stopped by heating to 65ºC for ten minutes. The tube was then placed on ice.

In Vitro Transcription

12.5 µl of cDNA was incubated at 37ºC for ten minutes to re-anneal the strands. The T7 Nucleotide Mix and 10X T7 Reaction Buffer were thawed at room temperature. The 10X T7 Reaction Buffer was thoroughly vortexed prior to using. To each cDNA sample, 8 µl T7 Nucleotide Mix, 2.5 µl 10X T7 Reaction Buffer, and 2 µl T7 Enzyme Mix was added at room temperature. This reaction was gently mixed and microfuged prior to incubating at 37ºC in a thermocycler with a heated lid for 16 hours.

Purification of senseRNA

The senseRNA was purified using the RNeasy MinElute Kit (Qiagen catalog number 74204) following Qiagen's protocol for RNA Cleanup. 75 µl of RNase-free water was added to the senseRNA for a final volume of 100 µl. This was accomplished by first transferring the 25 µl senseRNA mix into a new 1.5 ml microtube. Then 75 µl RNase-free water was placed into the PCR reaction tube utilized for the overnight incubation, pipetted up and down a few times to wash the sides of the tube, and the entire solution pipetted up and then placed into the 1.5 ml microtube and mixed thoroughly with the senseRNA.

350 µl Buffer RLT was placed into the tube and mixed thoroughly by pipetting. Then 250 µl 100% ethanol was added and mixed by pipetting. The entire 700 µl was applied to an RNeasy MinElute Spin Column in a 2 ml collection tube and was centrifuged at 10,000 rpm for 15 seconds. The flow-through was discarded, and the column transferred into a new 2 ml collection tube. 500 µl Buffer RPE was placed onto the spin column, the lid closed gently, and the column centrifuged at 10,000 rpm for 15 seconds for washing. The flow-through was discarded, and 500 µl 80% ethanol was

added to the column. The lid was closed gently, and the tube centrifuged for 2 minutes at 10,000 rpm to dry the silica-gel membrane. Afterwards, the flow-through and collection tube were discarded.

The spin column was transferred into a new 2 ml collection tube, the cap opened, and then centrifuged at full speed (13,000 rpm) for 5 minutes. The flow-through and collection tube were discarded. The spin column was transferred into a 1.5 ml collection tube, and 14 µl RNase-free water was pipetted directly onto the membrane for elution. The tube was left at room temperature for ten minutes, and then it was centrifuged for 1 minute at 13,000 rpm to elute the sample (approximately 12 µl).

Poly (A) Tailing of senseRNA

The volume of senseRNA was brought to 15.5 µl with nuclease free water. The ATP mix was diluted in 1mM Tris pH 8.0 for each tailing reaction, according to the following formula:

µg of senseRNA 50 = Dilution of ATP Mix

A 1:5 dilution of the PAP Enzyme in 1X PAP Buffer was made by first preparing a 1X PAP Buffer, by adding 2 µl of 5X PAP Buffer to 8 µl nuclease free water. This dilution was vortexed briefly and microfuged. 1 µl of the PAP Enzyme was added to 4 µl of 1X PAP Buffer, tapped gently to mix, microfuged briefly, and then placed on ice until ready to use.

For each reaction, 5.0 µl 5X PAP Buffer, 2.5 µl MnCl2, 1.0 µl ATP Mix, and 1.0 µl diluted PAP Enzyme were added to the 15.5 µl of sense RNA, for a total volume of 25 µl. The reaction was mixed gently, microfuged, and incubated at 37ºC for 15 minutes. The reaction was stopped by adding 3 µl of 0.5M EDTA, tapped to mix, and briefly

microfuged. The tailed senseRNA was purified using the RNeasy MinElute Kit following Qiagen's protocol for RNA Cleanup as previously described under "Purification of senseRNA." The senseRNA was stored at -80ºC.

Microarray Studies

Microarray Acquisition and Prehybridization

The microarrays utilized were purchased from the University of Texas Southwestern Medical Center Microarray Core Facility, Dallas, TX, USA. The microarrays were human synthetic polynucleotide (70-mer) microarrays spotted with the Human Oligo Set, Version 2, from Operon, representing 21,329 human genes.

Microarray Labeling and Hybridization

The microarrays were labeled utilizing the Genisphere 3DNA Array 900 Expression Array Detection Kit (Hatfield, PA, USA).

cDNA Synthesis from Total RNA. In a microtube, 2 µg total RNA (1-5 µl), 1 µl RT primer (1 pmole/µl Alexa Fluor 546 or Alexa Fluor 647), and nuclease free water to a final volume of 6 µl were combined to prepare the RNA-RT primer mix. The RNA-RT primer mix was mixed and microfuged briefly to collect the contents in the bottom of the tube. The RNA-RT primer mix was heated to 80°C for five minutes and then immediately transferred to ice for 2-3 minutes.

In a separate microtube, a reaction master mix (RMM) was prepared on ice. The

RMM was formulated to a final volume dependent on the number of cDNA syntheses that were to be conducted. Each cDNA synthesis required 4.5 µl of the RMM, which was composed of 2 µl 5X SuperScript II First Strand Buffer, 1 µl 0.1M DTT, 0.5 µl Superase-in RNAse Inhibitor, 0.5 µl deoxynucleotide Triphosphate Mix (dNTP) mix (10mM each dATP, dCTP, dGTP, dTTP), and 0.5 µl Superscript II enzyme, 200 units (Gibco, catalog #18064-014 – 10,000 Units @ 200U/µl). The RMM was gently mixed and microfuged briefly to collect the contents in the bottom of the tube. 4.5 µl of the RMM was added to the 6 µl of each RNA-RT primer mix, for a final volume of 10.5 µl. This was gently mixed (not vortexed) and incubated at 42ºC for four hours.

The cDNA synthesis reaction was stopped by adding 1 µl of 0.5M NaOH/50mM EDTA. This was followed by a 10-minute incubation at 65°C to denature the DNA/RNA hybrids and degrade the RNA. The reaction was neutralized with 1.2 µl of 1M Tris-HCl, pH 7.5.

cDNA Hybridization. A 2X Formamide-Based Hybridization Buffer was thawed at room temperature and resuspended by heating to 65-70°C for at least ten minutes or until completely resuspended. The components were vortexed to ensure they were resuspended evenly. The buffer was then microfuged for one minute.

For each array, a cDNA Hybridization Mix (cDHM) was prepared. This was composed of the 12.7 µl Pooled RNA cDNA reaction, the 12.7 µl Experimental RNA cDNA reaction, 2 µl LNA dT Blocker, 40 µl 2X Hybridization buffer, and 12.6 µl nuclease-free water, for a total of 80 µl cDHM. The cDHM was gently vortexed and

briefly microfuged, followed by a 10-minute incubation at 75-80°C, and then at the hybridization temperature (47°C) until loading the array.

The microarrays were prewarmed to the hybridization temperature of 47°C prior to being placed in a Telechem hybridization chamber. A 24 x 60 mm LifterSlip (Erie Scientific, Portmouth, NH, USA) was applied to each array. The cDHM was gently vortexed and briefly microfuged prior to being applied beneath the LifterSlip to a pre-warmed array. Any precipitate was left at the bottom of the microtube. 12 µl of 3X SSC was added at each end of the Telechem chamber. The arrays were incubated for 16 hours in a dark humidified chamber at 47°C.

Post cDNA Hybridization Wash. After hybridization, the slides were washed briefly several times to remove unbound cDNA molecules. The cover slips were removed by washing the arrays in 2X SSC, 0.2% SDS for 2-5 minutes at room temperature (or until cover slip floats off). This wash continued for a total time of 10-15 minutes. Next, the arrays were washed at room temperature in 2X SSC. The final wash was in 0.2 X SSC for 10-15 minutes at room temperature. Each array was then immediately transferred to a microarray high-speed centrifuge for rapid removal of the wash buffer (approximately 30 seconds).

3DNA Hybridization. The 3DNA Array 900 Capture Reagent for each Alexa Fluor was prepared by first thawing at room temperature 20 minutes in the dark. Then, the capture reagents are vortexed at the maximum setting for 3 seconds, followed by brief microfugation and incubation at 50-55°C for 10 minutes. Following the incubation, they

are vortexed at the maximum setting for 3-5 seconds, followed by another brief microfugation to collect the contents at the bottom. At the same time, the 2X Formamide-Based Buffer has been thawed and resuspended by heating to 70°C for at least 10 minutes or until completely resuspended. This was followed by vortexing and microfugation for one minute.

A 3DNA Hybridization Mix (3HM) was prepared for each array in separate microtubes. To 40 µl of 2X Formamide-Based Hybridization Buffer, 35 µl nuclease-free water, 2.5 µl Alexa Fluor 546 3DNA Capture Reagent, and 2.5 µl Alexa Fluor 647 3DNA Capture Reagent was added, to render a final 3HM volume of 80 µl. The 3HM was gently vortexed and briefly microfuged, followed by incubation for ten minutes at 75-80°C, and then at the hybridization temperature (45°C) until loading the array. The microarrays were prewarmed to the hybridization temperature of 45°C prior to being placed in a Telechem hybridization chamber. A 24 x 60mm LifterSlip was applied to each array. The 3HM was gently vortexed and briefly microfuged prior to being applied beneath the LifterSlip to a pre-warmed array. Any precipitate was left at the bottom of the microtube. 12 µl of 3X SSC was added to each end of the Telechem chamber. The arrays were incubated for three hours in a dark humidified chamber at 45°C.

Post 3DNA Hybridization Wash. After hybridization, the slides were washed briefly several times to remove unbound cDNA molecules. The coverslips were removed by washing the arrays in 2X SSC, 0.2% SDS for 2-5 minutes at room temperature (or until cover slip floats off). This wash continued for a total time of 10-15 minutes. Next, the arrays were washed at room temperature in 2X SSC. The final wash was in 0.2 X SSC for 10-15 minutes at room temperature. Each array was then immediately

transferred to a microarray high-speed centrifuge for rapid removal of the wash buffer. The slides were immediately transferred to a light-protective slide box, taking care not to touch the array surface.

Microarray Scanning

The microarrays were scanned and hybridization signals measured with the ScanArray Express (PerkinElmer, Boston, MA, USA).

Microarray Image Analysis and Spot Quanitification

Scanned images were analyzed utilizing GenePix Pro 4.0. Individual images were uploaded into GenePix Pro, as well as the GenePix Array List (GAL) file specific for the arrays utilized in the experiments. Each image was visually analyzed block-by-block, and features flagged that were considered inappropriate for inclusion in statistical analysis, due to either increased background intensity, increased fluorescence secondary to damage or a foreign particle on the array, or overlap of the feature with an adjacent feature(s).

Data Pre-Processing and Normalization Using Genepix Pro Auto-Processor

Pre-processing of data was accomplished using GenePix Autoprocessor (GPAP) (http://darwin.biochem.okstate.edu/gpap/) (Ayoubi et al., unpublished results). This analysis included: 1) removal of data where the fluorescence signal intensity in both channels was less than the background plus two standard deviations; 2) removal of data points where the signal was less than 200 Relative Fluorescence Units in both the

channels; 3) removal of bad quality spots flagged during processing of the image using GenePix Pro; 4) log 2 transformation of the background subtracted Alexa 647/Alexa 546 median ratios. Following pre-processing, the expression results were normalized using print tip LOWESS normalization and a t-statistic, p-value, and B-statistic were calculated for each gene based on the Empirical Bayes approach using the R-project statistical environment (http://www.r-project.org/) with the Bioconductor (http://www.bioconductor.org/) and Limma packages through the GPAP web site (http://darwin.biochem.okstate.edu/gpap3/) (Ayoubi et al., unpublished results).

Selection of Significant Genes

Results from GPAP were imported into a Microsoft Excel file and genes of interest selected based upon the following parameters. Log2 ratios were converted to absolute values, and those genes with an absolute log2 ratio of less than one (fold-change less than two), were removed. The list was then resorted according to the number of spots/replicates utilized in the GPAP processing. For the eutopic endometrium studies, only genes with at least 8 out of 12 spots utilized for statistical analysis, or the equivalent (i.e. $\geq$ 16/24), were retained. For the normal pelvic peritoneum studies, only genes with at least 6 out of 8 spots used in GPAP were retained. The list was then resorted based upon p-value, and only genes with a p-value <0.01 were retained. The remaining genes were those considered to demonstrate significant differential gene expression between women with and without endometriosis and subject to further analysis.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

cDNA Synthesis

cDNA was synthesized for each total RNA utilized in the microarray experiments, utilizing the ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis (Invitrogen, catalog # 11146-024). Therefore, individual cDNAs were made for each of the experimental RNA samples, as well as for the pooled eutopic endometrium and pooled normal pelvic peritoneum samples. The ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis includes ThermoScript™ RT (15 units/µl), oligo$(dT)_{20}$ (50 µM), random hexamers (50 ng/µl), 5X cDNA synthesis buffer, 0.1 M DTT, 10 mM dNTP mix, RNaseOUT™ RNase inhibitor (40 units/µl), *E. coli* RNase H (2 units/µl), and diethylpyrocarbonate (DEPC)-treated water.

In a 0.2 ml tube, 0.5 µl oligo$(dT)_{20}$ primer, 0.5 µl random hexamers, 2.0 µl dNTP, total RNA (100 ng to 2 µg) were combined, with DEPC-treated water added to a final volume of 12 µl. They were mixed well by pipetting, microfuged briefly, and then the RNA and primers were denatured by incubating at 65°C for 5 minutes. Afterwards, they were microfuged briefly and placed on ice. A master mix was prepared on ice with 4 µl 5x cDNA Synthesis Buffer (vortexed for five seconds just prior to use), 1 µl 0.1 M DTT, 1 µl RNaseOUT™, 1 µl DEPC-treated water, and 1 µl ThermoScript™ RT for each reaction plus enough of each component for one additional reaction to compensate for pipetting errors. 8 µl of master reaction mix was pipetted into each reaction tube on ice. The tubes were incubated at 25°C for 10 minutes, followed by 50 minutes at 55°C. The reaction was terminated by incubating at 85°C for 5 minutes. 1 µl of RNase H was added

to each reaction, followed by an incubation at 37°C for 20 minutes. The cDNA synthesis reactions were stored at -20°C until use in RT-PCR reactions.

Primer Design

Primers were designed utilizing Invitrogen's OligoPerfect™ Designer (http://www.invitrogen.com/content.cfm?pageid=9716). Forward and reverse primer sequences were entered into NCBI's BLAST Queue for "short, nearly exact matches" (nucleotide-nucleotide BLAST) and limited to the Homo sapiens database for confirmation of the forward and reverse primer pair being specific the gene of interest. Primers were ordered from the Midland Certified Reagent Company (Midland, Texas) or Invitrogen (Tables I and II).

Quantitative Reverse Transcription-Polymerase Chain Reaction

PCR reaction mixes were composed of 15.8 μl sterile water, 2.5 μl 10X PCR Buffer (Invitrogen), 1 μl Magnesium Chloride (Invitrogen), 0.5 μl dNTP mix (Invitrogen, catalog #18427-013), 0.1 μl 30% BSA (Sigma-Aldrich), 2.5 μl Sybr Green (Molecular Probes, Inc, Eugene, Oregon) (working stock made fresh daily by adding 0.25 μl Sybr Green to 500 μl sterile water for final concentration in each reaction of 1:20,000), 0.1 μl Platinum® Taq DNA polymerase (Invitrogen, catalog # 10966-026), 0.5 μl template cDNA, and 1 μl (20 pM) each of forward and reverse gene primers, for a total reaction volume of 25 μl.

A Cepheid Smart Cycler (Sunnyvale, California) was utilized for the PCR reactions. Each reaction began with a 95ºC hot start for 180 seconds. Samples were

subjected to 40 amplification cycles, which included a denaturing step at 95ºC for 15 seconds, a primer dependent annealing step at 58-64ºC for 45 seconds, and an extension step at 72ºC for 45 seconds. Optical data was collected during the annealing step. A melting curve was performed at the end of every run to ensure a single product was amplified.

Analysis

Optical Data from each RT-PCR experiment were transferred into a Microsoft Excel worksheet. The raw optics data were first converted into a logarithmic format, then gene to control (glyceraldeheyde-3-phosphate dehydrogenase, *GAPDH*) ratios calculated for each run and multiplied by one thousand to determine product abundance. An average for each sample was calculated based upon the number of PCR experiment replicates (typically three). The average of each experimental subject's abundance was divided by that of the pooled normals to determine fold-change. The average fold-change was calculated by adding all experimental subjects fold-change values and dividing by six (eutopic endometrium experiments) or three (normal pelvic peritoneum experiments). Replicates were excluded only when the optics data for the control gene (*GAPDH*) or gene of interest indicated an error within the RT-PCR reaction.

Table I. Primers Utilized in Validation Studies of Eutopic Endometrium Microarray Results.

Primer Name	Sequence	Size of PCR band
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward	5’-TGATGACATCAAGAAGGTGGTGAAG-3’	240 base pairs
GAPDH, reverse	5’-TCCTTGGAGGCCATGTGGGCCAT-3’	
Homo sapiens BCOO9393, forward	5’-CCCTCATCGTGTCAAGTCAA-3’	237 base pairs
Homo sapiens BCOO9393, reverse	5’-AGGCATCAAAGTTGGCAAAG-3’	
Growth arrest and DNA-damage-inducible, beta (GADD45B), forward	5'-CAG AAG ATG CAG ACG GTG AC-3'	214 base pairs
GADD45B, reverse	5'-TGT CAC AGC AGA AGG ACT GG-3'	
Mammoglobin (MGB1), forward	5’-CTC CCA GCA CTG CTA CGC AGG CTC-3’	201 base pairs
MGB1, reverse	5’-CAC CTC AAC ATT GCT CAG AGT TTC ATC CG-3’	
Thrombospondin 3 (THBS3), forward	5’-ACT GCC TTT TGA CAC CCA AC-3’	179 base pairs
THBS3, reverse	5’-ATT GTC ACA GGC ATC ACC AA-3’	
G2 & S phase expressed 1 (GTSE1), forward	5’-GGG ATG TTC TCC CTG ACA AA-3’	232 base pairs
GTSE1, reverse	5’-GAT TTA GCT TTG CCC ACT GC-3’	

Table II. Primers Utilized in Validation Studies of Normal Pelvic Peritoneum Microarray Results.

Primer Name	Sequence	Size of PCR band
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forward	5'-TGATGACATCAAGAAGGTGGTGAAG-3'	240 base pairs
GAPDH, reverse	5'-TCCTTGGAGGCCATGTGGGCCAT-3'	
Suppressor of cytokine signaling 2 (SOCS2) forward	5'-AAC AGC AGT AAA TAA ACA CTT TGA A-3'	249 base pairs
SOCS2, reverse	5'-GCA ACT CAA TCC CCG TTA AT-3'	
Minichromosome maintenance deficient 5, cell division cycle 46 (MCM5), forward	5'-CTA CAC TGG ACT CAT GGA CTC G-3'	213 base pairs
MCM5 reverse	5'-ATT AAC GGA TGC CAA AAG CA-3'	
A20-binding inhibitor of NF-kappaB activation 2 (TNIP2), forward	5'-AAG TGT GCT TTA ACT TCC GGC-3'	209 base pairs
TNIP2, reverse	5'-GGG AGT CAC TTG TAT GGT CCC-3'	

CHAPTER IV

RESULTS

Participant Characteristics

Seven women with endometriosis and seven women without disease were utilized in the microarray studies. The average age of the women with endometriosis was 26.43 (SD ± 5.83), and the average age of the normal participants was 26.57 (SD ± 5.62). Three women had Stage I endometriosis, two women had Stage II, and two women had Stage III. All endometriosis participants had a history of pelvic pain, with 6 of 7 complaining of dysmenorrhea, 5 of 7 having dyspareunia, and three complaining of pain with bowel movements. None of the normal participants endorsed a history of pelvic pain. In regards to infertility, one woman with endometriosis and one without admitted to this, however, the women without endometriosis had been pregnant previously, and stated that her infertility involved her last attempt at conceiving taking 23 months. Three of seven women with endometriosis had a family member with endometriosis, in contrast to only one normal participant.

Three women without endometriosis were utilized for the normal pool of eutopic endometriosis (average age 25.67, SD ± 6.43). Six women with endometriosis were utilized for the endometriosis experiments (mean age 26.00, SD ± 6.26). Normal pelvic peritoneum from four normal participants (mean age 27.25, SD ± 5.85) and from three

women with endometriosis (mean age 24.00, SD ± 5.57) was utilized in the second series of microarray experiments.

Differential Gene Expression Studies of Eutopic Endometrium in Women with and without Endometriosis

756 genes were found to be significantly up- or down-regulated in the eutopic endometrium of women with endometriosis versus those without (Table III). 352 genes were up-regulated greater than 2-fold with a p-value <0.01, and 404 were down-regulated greater than two-fold. The most up-regulated gene was Kelch-like 1 (NM_020866) with a fold change of +36.429 (log2 ratio 5.187, SD 1.336). Nine replicates were utilized in the analysis, with a p-value of 6.83×10^{-6}. The gene most down-regulated, with a fold-change of -113.300 (log2 ratio -6.824, SD 0.935) was Homo sapiens HIV-1 Rev binding protein-like (BC009393). Ten replicates were utilized in its analysis, rendering a p-value of 4.35×10^{-10}.

Table III. Log Ratio Changes of Gene Expression in Eutopic Endometrium of Women with Endometriosis versus Without.

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_020866	Kelch-like 1 (Drosophila)	KLHL1	36.429	5.187	1.336	0.284	9.936	6.83E-06	8.173	9
NM_015887	Putative peroxisome microbody protein 175.1	LOC51051	23.703	4.567	1.486	0.416	7.526	1.19E-04	4.294	11
AL050175	Homo sapiens mRNA		23.296	4.542	1.503	0.331	11.410	3.18E-06	9.269	11
AK021930	Homo sapiens cDNA FLJ11868 fis		21.963	4.457	0.680	0.157	15.310	1.30E-07	13.918	9
NM_032293	GTPase activating Rap/RanGAP domain-like	GARNL3	20.692	4.371	0.520	0.124	21.900	4.62E-09	18.736	11
NM_006732	FBJ murine osteosarcoma viral oncogene homolog B	FOSB	20.210	4.337	1.278	0.283	11.220	2.16E-06	9.800	8
AB014542	Trinucleotide repeat containing 15	TNRC15	15.835	3.985	1.399	0.351	11.100	2.36E-06	9.574	12
AK025643	Homo sapiens cDNA: FLJ21990 fis		15.671	3.970	1.308	0.302	8.866	1.97E-05	6.662	11
NM_005738	ADP-ribosylation factor-like 4A	ARL4	14.035	3.811	1.321	0.347	11.230	2.16E-06	9.722	12
NM_005252	V-fos FBJ murine osteosarcoma viral oncogene homolog	FOS	13.823	3.789	0.812	0.185	8.624	2.54E-05	6.405	8
AK022048	Homo sapiens cDNA FLJ11986 fis		13.813	3.788	1.638	0.644	5.353	1.68E-03	0.831	8
NM_020433	Junctophilin 2	JPH2	13.260	3.729	1.528	0.405	9.631	9.39E-06	7.801	9
NM_017947	Molybdenum cofactor sulfurase	HMCS	12.355	3.627	1.352	0.373	10.450	4.14E-06	8.773	12
NM_001249	Ectonucleoside triphosphate diphosphohydrolase 5	ENTPD5	12.227	3.612	0.535	0.149	23.390	6.26E-09	18.212	10
NM_017936	Homo sapiens KIAA2010	KIAA2010	11.984	3.583	1.040	0.290	13.310	4.76E-07	12.006	12
S66666	P53		11.852	3.567	0.709	0.214	11.480	1.74E-06	10.081	8
NM_005420	Sulfotransferase family 1E, estrogen-preferring, member 1	STE	11.672	3.545	1.777	0.501	7.804	6.24E-05	5.082	12
AB051514	KIAA1727 protein		11.400	3.511	1.347	0.382	10.180	5.40E-06	8.460	11
AB037715	KIAA1294 protein		11.377	3.508	0.694	0.209	14.580	1.95E-07	13.240	11
NM_003215	Tec protein tyrosine kinase	TEC	11.035	3.464	0.939	0.271	14.200	2.51E-07	12.882	12

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
AF116656	Homo sapiens PRO1167 mRNA		10.793	3.432	2.244	0.723	6.117	8.23E-04	1.880	9
AK055405	Homo sapiens cDNA FLJ30843 fis		10.440	3.384	1.628	0.475	8.040	6.90E-05	5.151	8
AK055061	Homo sapiens cDNA FLJ30499 fis		10.225	3.354	0.551	0.154	15.800	9.85E-08	14.336	9
AK057198	Homo sapiens cDNA FLJ32636 fis		10.218	3.353	1.928	0.563	6.834	1.94E-04	3.617	9
AI871129	ESTs		9.911	3.309	0.644	0.192	17.760	2.90E-08	15.909	10
NM_006174	Neuropeptide Y receptor Y5	NPY5R	9.680	3.275	2.005	0.671	6.800	2.67E-04	3.202	11
NM_013315	Transmembrane phosphatase with tensin homology	TPTE	9.640	3.269	1.213	0.368	9.281	1.30E-05	7.360	8
AF330042	Homo sapiens Alu repeat mRNA sequence		9.626	3.267	1.529	0.467	8.452	3.03E-05	6.123	10
NM_007281	Scrapie responsive protein 1	SCRG1	9.468	3.243	0.727	0.237	13.270	4.87E-07	11.982	10
NM_000635	Regulatory factor X, 2 (influences HLA class II expression)	RFX2	9.383	3.230	1.321	0.405	9.520	1.03E-05	7.623	10
AK055220	Homo sapiens cDNA FLJ30658 fis		9.370	3.228	1.944	0.587	6.493	2.94E-04	3.038	9
NM_001145	Angiogenin, ribonuclease, Rnase A family, 5	RNASE4	9.088	3.184	1.403	0.396	6.807	2.01E-04	3.467	11
AK022029	Homo sapiens cDNA FLJ11967 fis		8.821	3.141	1.069	0.340	11.360	1.90E-06	9.882	12
NM_018321	Brix domain containing 2	BRIX	8.815	3.140	0.984	0.361	7.092	1.42E-04	4.044	8
NM_003764	Syntaxin 11	STX11	8.815	3.140	1.505	0.469	8.099	4.53E-05	5.666	8
AB033104	Serine/threonine kinase 36 (fused homolog, Drosophila)	STK36	8.562	3.098	0.599	0.206	13.340	4.75E-07	12.050	10
NM_017765	PQ loop repeat containing 2	PQLC2	8.550	3.096	0.708	0.217	13.550	3.99E-07	12.244	11
BC014396	Copine IV	CPNE4	8.386	3.068	1.219	0.439	7.756	6.60E-05	5.008	11
AK054975	Homo sapiens cDNA FLJ30413 fis		8.374	3.066	0.738	0.237	14.640	1.93E-07	13.319	9
AK054969	Homo sapiens cDNA FLJ30407 fis		8.276	3.049	0.953	0.312	13.610	3.83E-07	12.323	20
AK024118	Homo sapiens cDNA FLJ14056 fis		8.208	3.037	0.680	0.220	14.910	1.69E-07	13.566	9
NM_014797	Zinc finger and BTB domain containing 24	ZBTB24	7.994	2.999	1.532	0.601	7.607	1.08E-04	4.448	19

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
BC012136	ISL2 transcription factor, LIM/homeodomain (islet-2)	ISL2	7.890	2.980	1.273	0.426	9.250	1.33E-05	7.256	10
AK026419	Homo sapiens cDNA: FLJ22766 fis		7.835	2.970	0.826	0.305	9.758	8.20E-06	7.940	9
AB051488	KIAA1701 protein		7.717	2.948	1.536	0.516	7.425	9.53E-05	4.514	11
NM_024753	Tetratricopeptide repeat domain 21B	TTC21B	7.600	2.926	1.371	0.512	9.865	1.19E-05	7.507	11
AL049650	Transglutaminase 3-like	TGM3L	7.444	2.896	0.976	0.331	11.040	2.45E-06	9.575	9
NM_030820	Collagen, type XXI, alpha 1	COL21A1	7.200	2.848	1.083	0.401	9.247	1.33E-05	7.224	21
NM_007215	Polymerase (DNA directed), gamma 2, accessory subunit	POLG2	7.106	2.829	1.043	0.410	10.660	3.48E-06	9.084	21
AB020691	KIAA0884 protein, GTPase activating Rap/RanGAP domain-like 1	GARNL1	6.979	2.803	0.860	0.303	12.010	1.17E-06	10.660	10
BC013114	Homo sapiens aarF domain containing kinase 4		6.960	2.799	1.058	0.375	9.918	1.14E-05	7.599	9
AL137707	Homo sapiens mRNA		6.921	2.791	0.453	0.150	12.660	7.25E-07	11.343	10
AF339803	Homo sapiens clone IMAGE:246773		6.797	2.765	1.144	0.401	8.629	2.53E-05	6.412	9
NM_014810	Centrosome-associated protein 350kDa	CAP350	6.779	2.761	1.004	0.361	10.510	3.89E-06	8.873	11
NM_013416	Neutrophil cytosolic factor 4, 40kDa	NCF4	6.671	2.738	1.236	0.449	8.316	5.19E-05	5.472	10
AK054623	Homo sapiens cDNA FLJ30061 fis		6.561	2.714	1.386	0.505	7.581	7.91E-05	4.831	9
NM_004617	Transmembrane 4 superfamily member 4	TM4SF4	6.525	2.706	0.737	0.268	13.000	5.95E-07	11.717	10
AL137361	Homo sapiens genomic DNA	DKFZP434C0826	6.467	2.693	1.251	0.456	8.123	4.42E-05	5.633	10
NM_002666	Perilipin	PLIN	6.431	2.685	0.643	0.261	10.650	3.48E-06	9.051	10
NM_024845	Hypothetical protein FLJ14154	FLJ14154	6.360	2.669	1.025	0.384	10.050	6.08E-06	8.273	12
NM_016426	G-2 and S-phase expressed 1	GTSE1	6.347	2.666	1.067	0.396	9.620	9.47E-06	7.757	10
Z00008	Immunoglobulin kappa variable 1D-8	IGKV1D-8	6.333	2.663	0.532	0.198	17.320	3.96E-08	15.574	10
NM_032447	Fibrillin 3	FBN3	6.281	2.651	0.743	0.277	12.410	8.58E-07	11.076	11
AL136945	Zinc finger protein 265	ZNF265	6.238	2.641	1.166	0.492	7.056	1.48E-04	3.882	11

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_012204	General transcription factor IIIC, polypeptide 4, 90kDa	GTF3C4	6.097	2.608	1.077	0.407	9.066	1.61E-05	7.001	10
AK024867	Homo sapiens cDNA: FLJ21214 fis		6.050	2.597	1.262	0.473	7.756	6.60E-05	5.105	9
NM_003407	Zinc finger protein 36, C3H type, homology (mouse)	ZFP36	5.938	2.570	0.916	0.323	8.236	3.90E-05	5.771	10
AL359599	Homo sapiens genomic DNA		5.938	2.570	0.421	0.163	19.530	2.80E-08	16.011	10
NM_014370	Serine/threonine kinase 23	STK23	5.934	2.569	1.107	0.495	6.517	2.85E-04	3.005	10
AK023446	Homo sapiens cDNA FLJ13384 fis, aminoadipate-semialdehyde synthase	AASS	5.889	2.558	1.055	0.412	9.374	1.19E-05	7.369	12
NM_005701	RNA, U transporter 1	RNUT1	5.776	2.530	0.707	0.279	13.560	3.99E-07	12.250	12
NM_004414	Down syndrome critical region gene 1	DSCR1	5.756	2.525	0.801	0.283	7.581	7.91E-05	4.831	8
AK055705	Homo sapiens cDNA FLJ31143 fis		5.748	2.523	1.101	0.438	9.070	1.61E-05	7.070	8
AK055210	Homo sapiens cDNA FLJ30648 fis		5.744	2.522	1.097	0.430	8.873	1.96E-05	6.729	10
AB058717	KIAA1814 protein		5.708	2.513	1.120	0.443	8.770	2.17E-05	6.584	10
AK001044	Homo sapiens cDNA FLJ10182 fis		5.653	2.499	0.588	0.253	11.710	1.43E-06	10.300	10
NM_002045	Growth associated protein 43	GAP43	5.560	2.475	1.320	0.529	7.033	1.51E-04	3.988	8
NM_030751	Transcription factor 8 (represses interleukin 2 expression)	TCF8	5.560	2.475	1.411	0.560	6.772	2.08E-04	3.475	10
AF131846	Homo sapiens clone 25028 mRNA sequence		5.449	2.446	1.141	0.465	8.345	3.44E-05	5.934	11
NM_014757	Mastermind-like 1 (Drosophila)	MAML1	5.393	2.431	0.954	0.392	9.814	7.76E-06	7.961	12
NM_015996	CGI-40 protein, SID1 transmembrane family, member 2	SIDT2	5.367	2.424	1.028	0.475	7.263	1.15E-04	4.254	10
AB033114	AT2 receptor-interacting protein 1	MTSG1	5.359	2.422	1.241	0.509	7.583	7.90E-05	4.767	11
AK025766	Homo sapiens cDNA: FLJ22113 fis		5.337	2.416	0.981	0.448	7.705	6.93E-05	4.927	11
NM_006853	Kallikrein 11	KLK11	5.234	2.388	0.936	0.357	7.673	7.19E-05	4.877	11
AK057060	Homo sapiens cDNA FLJ32498 fis		5.205	2.380	1.510	0.626	6.239	4.14E-04	2.594	9

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_005415	Solute carrier family 20 (phosphate transporter), member 1	SLC20A1	5.177	2.372	1.303	0.545	6.938	2.28E-04	3.498	8
NM_002618	Peroxisome biogenesis factor 13	PEX13	5.095	2.349	0.907	0.371	9.138	1.48E-05	7.163	8
NM_002888	Retinoic acid receptor responder (tazarotene induced) 1	RARRES1	5.091	2.348	0.981	0.400	8.554	2.73E-05	6.338	8
NM_006763	BTG family, member 2	BTG2	5.056	2.338	0.809	0.339	10.810	3.08E-06	9.315	8
NM_000583	Group-specific component (vitamin D binding protein)	GC	5.000	2.322	0.944	0.405	9.484	1.07E-05	7.546	11
NM_005032	Plastin 3 (T isoform)	PLS3	4.993	2.320	0.751	0.359	8.444	3.06E-05	6.051	11
NM_022145	Leucine zipper protein FKSG14	FKSG14	4.959	2.310	1.049	0.402	6.218	4.27E-04	2.448	11
AF063020	PC4 and SFRS1 interacting protein 2	PSIP2	4.884	2.288	1.156	0.503	7.683	7.12E-05	4.924	11
AK055656	Homo sapiens cDNA FLJ31094 fis	LOC219688	4.843	2.276	0.448	0.211	12.400	8.58E-07	11.104	8
U79280	Human clone 23575 mRNA	PIPPIN	4.837	2.274	1.521	0.656	5.941	6.19E-04	2.107	8
NM_022496	ARP6 actin-related protein 6 homolog	ACTR6	4.830	2.272	0.741	0.357	8.967	1.80E-05	6.804	11
U72518	Human destrin-2 pseudogene mRNA		4.813	2.267	1.021	0.448	8.669	2.42E-05	6.438	10
NM_015450	Protection of telomeres 1	POT1	4.787	2.259	1.051	0.474	9.374	1.19E-05	7.370	21
AK056642	Homo sapiens cDNA FLJ32080 fis		4.783	2.258	1.583	0.696	5.640	9.40E-04	1.476	10
NM_014432	Interleukin 20 receptor, alpha	IL20RA	4.754	2.249	0.902	0.401	5.082	2.03E-03	0.356	10
AL080077	Homo sapiens mRNA		4.734	2.243	0.910	0.401	9.043	1.65E-05	6.940	11
AL049357	Homo sapiens mRNA		4.714	2.237	1.058	0.473	8.224	3.95E-05	5.785	10
AK027772	Homo sapiens cDNA FLJ14866 fis	C13orf10	4.611	2.205	1.122	0.501	7.362	1.03E-04	4.447	10
NM_006551	Lipophilin B (uteroglobin family member)	SCGB1D2	4.557	2.188	1.693	0.774	5.053	2.12E-03	0.301	12
NM_003362	Uracil-DNA glycosylase	UNG	4.554	2.187	0.608	0.274	12.900	6.07E-07	11.616	10
NM_004599	Sterol regulatory element binding transcription factor 2	SREBF2	4.510	2.173	1.449	0.663	5.868	6.88E-04	1.857	11
NM_001371	Dynein	DNAH8	4.503	2.171	0.772	0.407	6.989	1.59E-04	3.772	11

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NM_002634	Prohibitin	PHB	4.485	2.165	0.439	0.226	8.939	1.84E-05	6.793	10
NM_002411	Mammaglobin 1	SCGB2A2	4.469	2.160	1.618	0.740	5.246	1.61E-03	0.740	10
AJ420568	DKFZP586D0623 protein		4.438	2.150	1.060	0.493	7.848	5.96E-05	5.150	12
BC007594	Nucleolar protein 3 (apoptosis repressor), Homo sapiens, Similar to RIKEN cDNA 4931428F04 gene		4.405	2.139	0.911	0.484	7.052	1.48E-04	3.908	10
AK021665	Homo sapiens cDNA FLJ11603 fis		4.392	2.135	1.002	0.463	8.065	4.70E-05	5.546	10
NM_003083	Small nuclear RNA activating complex	SNAPC2	4.368	2.127	0.726	0.348	9.271	1.31E-05	7.286	10
NM_052815	Immediate early response 3	IER3	4.323	2.112	1.325	0.625	6.243	4.12E-04	2.527	11
AK055015	Homo sapiens cDNA FLJ30453 fis		4.317	2.110	1.148	0.528	6.685	2.32E-04	3.327	10
NM_013288	DNA binding protein for surfactant protein B	HUMBIN DC	4.314	2.109	1.442	0.750	5.872	8.58E-04	1.659	11
NM_014465	Sulfotransferase family	SULT1B1	4.284	2.099	1.294	0.606	6.484	2.99E-04	3.063	8
NM_025040	Hypothetical protein FLJ21941	FLJ21941	4.216	2.076	1.020	0.398	4.979	2.35E-03	0.273	8
AK056177	Homo sapiens cDNA FLJ31615 fis		4.211	2.074	1.244	0.604	6.463	3.07E-04	2.911	11
NM_000917	Procollagen-proline	P4HA1	4.202	2.071	1.238	0.598	6.501	2.91E-04	2.944	12
NM_053278	Homo sapiens G protein-coupled receptor 102	GPR102	4.187	2.066	1.104	0.533	7.280	1.12E-04	4.281	11
NM_005442	Eomesodermin homolog (Xenopus laevis)	EOMES	4.184	2.065	0.821	0.362	7.609	7.71E-05	4.776	11
NM_003435	Zinc finger protein 134 (clone pHZ-15)	ZNF134	4.144	2.051	0.511	0.269	10.810	3.08E-06	9.222	11
AL353937	KIAA1280 protein	KIAA1280	4.135	2.048	1.291	0.624	6.397	3.35E-04	2.912	8
NM_005201	Chemokine (C-C motif) receptor 8	CCR8	4.124	2.044	0.951	0.463	8.300	3.62E-05	5.866	11
NM_005245	FAT tumor suppressor homolog 1 (Drosophila)	FAT	4.112	2.040	0.790	0.430	9.777	8.04E-06	7.912	23
AK021484	Homo sapiens cDNA FLJ11422 fis		4.101	2.036	0.713	0.328	11.730	1.42E-06	10.311	23
NM_005077	Transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	TLE1	4.070	2.025	0.594	0.330	8.006	5.03E-05	5.455	9

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NM_000247	MHC class I polypeptide-related sequence	MICA	4.045	2.016	1.348	0.663	5.818	7.39E-04	1.765	11
NM_022829	Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3	SLC13A3	4.028	2.010	0.901	0.446	8.541	2.76E-05	6.222	11
AL049218	Homo sapiens mRNA	FLJ11712	4.022	2.008	1.754	0.871	4.599	4.10E-03	-0.463	9
AK022346	Homo sapiens cDNA FLJ12284 fis		3.953	1.983	0.625	0.353	8.334	3.48E-05	5.949	9
NM_033226	ATP-binding cassette, sub-family C, member 12	ABCC12	3.926	1.973	0.915	0.464	8.291	3.65E-05	5.824	12
BC012458	Homo sapiens		3.907	1.966	0.523	0.262	12.390	8.60E-07	11.073	10
NM_001220	Calcium/calmodulin-dependent protein kinase (CaM kinase) II beta	CAMK2B	3.848	1.944	1.858	0.926	4.222	7.12E-03	-1.161	8
NM_002061	Glutamate-cysteine ligase	GCLM	3.834	1.939	0.686	0.398	7.442	9.30E-05	4.543	10
NM_002229	Jun B proto-oncogene	JUNB	3.821	1.934	0.952	0.492	7.827	6.09E-05	5.118	12
NM_003408	Zinc finger protein 37 homolog (mouse)	ZFP37	3.803	1.927	1.327	0.563	4.289	6.45E-03	-1.117	9
NM_003059	Solute carrier family 22 (organic cation transporter), member 4	SLC22A4	3.789	1.922	0.998	0.517	7.450	9.23E-05	4.555	11
NM_012446	Single-stranded DNA binding protein 2	SSBP2	3.789	1.922	1.985	1.363	4.013	9.62E-03	-1.707	20
AF172398	Junctional adhesion molecule 1	F11R	3.782	1.919	0.536	0.279	13.200	5.12E-07	11.887	12
NM_006182	Discoidin domain receptor family	DDR2	3.771	1.915	1.171	0.607	6.405	3.32E-04	2.846	10
NM_022832	Hypothetical protein FLJ12552	USP46	3.769	1.914	0.997	0.517	7.368	1.02E-04	4.424	11
AL157425	Homo sapiens cDNA FLJ12764 fis		3.755	1.909	0.808	0.414	8.766	2.17E-05	6.609	9
BC015239	Homo sapiens		3.714	1.893	1.021	0.528	7.150	1.33E-04	4.140	9
AK055946	Homo sapiens cDNA FLJ31384 fis		3.665	1.874	0.653	0.432	5.371	1.37E-03	0.976	9
AY039760	Homo sapiens V-ATPase G3 subunit (ATP6V1G3) mRNA	ATP6V1G3	3.648	1.867	1.173	0.622	6.222	4.24E-04	2.525	10
NM_015474	SAM domain and HD domain	SAMHD1	3.645	1.866	1.318	0.704	5.551	1.06E-03	1.275	11
AK055644	Homo sapiens cDNA FLJ14429 fis		3.640	1.864	0.928	0.486	6.950	3.10E-04	3.163	8
NM_003657	Breast carcinoma amplified sequence 1	BCAS1	3.618	1.855	0.633	0.333	10.660	3.48E-06	9.133	8

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
Y11918	H.sapiens IMAGE cDNA clone 26881		3.605	1.850	0.899	0.480	7.704	6.93E-05	4.957	11
AB014530	KIAA0630 protein	HIPK1	3.600	1.848	0.629	0.368	9.096	1.56E-05	6.987	11
NM_001338	Coxsackie virus and adenovirus receptor	CXADR	3.598	1.847	0.764	0.500	5.472	1.19E-03	1.128	10
AL137411	Homo sapiens mRNA		3.595	1.846	1.870	0.993	3.997	9.82E-03	-1.608	8
NM_005177	ATPase	ATP6V0A1	3.593	1.845	0.595	0.348	9.523	1.03E-05	7.572	11
AK025527	Homo sapiens cDNA: FLJ21874 fis	INSR	3.588	1.843	0.399	0.216	15.970	9.25E-08	14.479	11
NM_002034	Fucosyltransferase 5 (alpha (1,3) fucosyltransferase)	FUT5	3.570	1.836	1.407	0.732	4.969	2.37E-03	0.300	8
AK055794	Homo sapiens cDNA FLJ31232 fis		3.533	1.821	1.012	0.555	6.993	1.59E-04	3.811	11
AB025432	Delta sleep inducing peptide, Glucocorticoid-induced leucine zipper protein	GILZ	3.523	1.817	0.738	0.400	9.265	1.32E-05	7.306	9
NM_024594	Hypothetical protein FLJ12899	PANK3	3.519	1.815	0.964	0.529	7.041	2.04E-04	3.583	10
AK055962	ARG99 protein	ARG99	3.516	1.814	0.819	0.460	7.769	6.51E-05	5.090	17
AK057147	Homo sapiens cDNA FLJ32585 fis		3.509	1.811	0.481	0.266	12.470	8.13E-07	11.166	10
AK000801	Homo sapiens cDNA FLJ20794 fis		3.502	1.808	0.858	0.563	5.816	7.41E-04	1.799	9
AK055332	Homo sapiens cDNA FLJ30770 fis		3.494	1.805	0.779	0.426	8.521	2.79E-05	6.193	11
NM_025174	Hypothetical protein FLJ23040	FLJ20203	3.487	1.802	1.412	1.471	5.795	7.65E-04	1.843	16
AK023846	Hypothetical protein MGC3067	MGC3067	3.487	1.802	1.096	0.594	6.316	3.71E-04	2.730	9
AK026924	Homo sapiens cDNA: FLJ23271 fis		3.482	1.800	0.637	0.354	10.610	3.59E-06	8.981	12
NM_018282	Paraspeckle protein 1	PSP1	3.470	1.795	0.918	0.622	4.948	2.46E-03	0.135	10
NM_016468	Hypothetical protein	C14orf112	3.453	1.788	0.716	0.445	7.805	6.24E-05	5.219	17
AK056506	Homo sapiens cDNA FLJ31944 fis		3.444	1.784	0.813	0.452	8.304	3.62E-05	5.904	10
NM_007112	Thrombospondin 3	THBS3	3.422	1.775	1.100	0.571	6.030	5.48E-04	2.114	23
NM_006739	MCM5 minichromosome maintenance deficient 5, cell division cycle 45 (S. cerevisiae)	MCM5	3.420	1.774	0.444	0.283	9.047	1.64E-05	7.005	8
U18004	HSU18004 Homo sapiens cDNA		3.413	1.771	0.929	0.520	7.413	9.66E-05	4.565	9

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_024744	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 8	ALS2CR8	3.408	1.769	1.096	0.624	6.321	3.69E-04	2.739	9
AK024177	Homo sapiens cDNA FLJ14115 fis		3.406	1.768	1.538	0.865	4.546	4.43E-03	-0.610	10
AK055990	Homo sapiens cDNA FLJ31428 fis	DNAH9	3.392	1.762	0.929	0.527	7.304	1.10E-04	4.288	12
AK025151	Homo sapiens cDNA: FLJ21498 fis		3.373	1.754	1.234	0.708	5.597	1.00E-03	1.397	10
NM_033292	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	CASP1	3.368	1.752	1.257	0.787	5.621	1.20E-03	1.306	9
AL050353	Opa-interacting protein 2	OIP2	3.366	1.751	0.914	0.518	7.322	1.08E-04	4.349	11
AF057036	Collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase	COLQ	3.340	1.740	0.711	0.409	9.271	1.31E-05	7.229	12
NM_004417	Dual specificity phosphatase 1	DUSP1	3.338	1.739	1.470	0.838	4.621	3.97E-03	-0.501	11
AB001523	Transmembrane protein 1	TMEM1	3.336	1.738	0.866	0.493	7.667	7.25E-05	4.931	10
AK056032	Homo sapiens cDNA FLJ31470 fis		3.329	1.735	0.861	0.486	7.561	8.08E-05	4.800	9
NM_003275	Tropomodulin	TMOD1	3.304	1.724	0.794	0.467	8.480	2.94E-05	6.104	23
AB067501	Hypothetical protein FLJ14564		3.304	1.724	1.579	0.908	4.275	6.59E-03	-1.185	11
NM_002462	Myxovirus (influenza virus) resistance 1	MX1	3.299	1.722	1.488	0.855	4.600	4.09E-03	-0.462	9
NM_001523	Hyaluronan synthase 1	HAS1	3.278	1.713	0.872	0.604	5.532	1.09E-03	1.276	9
BC012204	Homo sapiens		3.260	1.705	0.967	0.558	6.705	2.26E-04	3.361	10
NM_014330	Protein phosphatase 1	PPP1R15A	3.258	1.704	0.449	0.257	12.000	1.17E-06	10.673	9
NM_002451	Methylthioadenosine phosphorylase	MTAP	3.254	1.702	0.907	0.533	7.307	1.09E-04	4.395	9
NM_016310	Polymerase (RNA) III (DNA directed) polypeptide K, 12.3 kDa	POLR3K	3.245	1.698	1.015	0.591	6.475	3.03E-04	2.968	10
AK025620	Non-kinase Cdc42 effector protein SPEC2	SPEC2	3.236	1.694	0.562	0.408	5.787	7.72E-04	1.709	10
NM_016932	Sine oculis homeobox homolog 2 (Drosophila)	SIX2	3.233	1.693	0.715	0.419	8.904	1.89E-05	6.773	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_005667	Zinc finger protein 103 homolog (mouse)	RNF103	3.231	1.692	0.929	0.549	7.009	1.56E-04	3.805	12
NM_000016	Acyl-Coenzyme A dehydrogenase	ACADM	3.227	1.690	1.144	0.656	5.800	7.59E-04	1.852	8
NM_001782	CD72 antigen	CD72	3.224	1.689	0.871	0.495	7.057	1.48E-04	4.027	8
NM_005664	Makorin	MKRN3	3.220	1.687	1.056	0.525	4.379	5.62E-03	-1.013	11
AL162040	Homo sapiens mRNA		3.207	1.681	0.658	0.386	9.364	1.19E-05	7.473	8
NM_015675	Growth arrest and DNA-damage-inducible, beta	GADD45B	3.171	1.665	0.600	0.360	10.360	4.47E-06	8.665	12
NM_021200	PH domain containing protein in retina 1	PLEKHB1	3.145	1.653	1.110	0.672	5.767	7.94E-04	1.638	12
NM_001500	GDP-mannose 4	GMDS	3.141	1.651	0.739	0.381	5.858	6.96E-04	1.875	9
NM_003388	Cytoplasmic linker 2	CYLN2	3.130	1.646	0.967	0.586	6.641	2.46E-04	3.253	10
NM_015984	Ubiquitin C-terminal hydrolase UCH37	UCHL5	3.123	1.643	1.182	1.049	5.985	5.84E-04	2.144	18
BC011883	Homo sapiens		3.108	1.636	0.857	0.525	7.349	1.04E-04	4.393	11
AK022337	Homo sapiens cDNA FLJ12275 fis		3.095	1.630	0.452	0.249	7.969	5.17E-05	5.367	10
AL137507	Homo sapiens mRNA		3.093	1.629	1.147	0.688	5.484	1.17E-03	1.229	9
AL390172	Homo sapiens cDNA: FLJ21270 fis	BCAT1	3.082	1.624	0.992	0.611	6.314	3.73E-04	2.618	12
NM_018083	Zinc finger protein 358	ZNF358	3.072	1.619	0.894	0.550	6.748	2.84E-04	3.118	10
AB032963	ATPase	ATP8B2	3.057	1.612	0.370	0.276	6.888	1.82E-04	3.670	9
AF155827	Helicase	HELLS	3.050	1.609	1.202	0.726	5.274	1.56E-03	0.880	8
NM_022916	Vacuolar protein sorting 33A (yeast)	VPS33A	3.046	1.607	1.361	0.808	4.594	4.13E-03	-0.429	8
AL133574	Homo sapiens mRNA		3.044	1.606	0.564	0.447	4.921	2.55E-03	0.082	10
AL049341	Kruppel-like zinc finger protein		3.042	1.605	0.674	0.361	6.162	4.59E-04	2.421	9
BC008442	Transmembrane 4 superfamily member 1	TM4SF1	3.040	1.604	1.461	0.904	4.295	6.40E-03	-1.145	11
AK057215	Homo sapiens cDNA FLJ32653 fis		3.017	1.593	0.547	0.341	10.520	3.87E-06	8.891	11
NM_006568	Cell growth regulatory with ring finger	CGRRF1	3.008	1.589	1.008	0.634	6.085	5.08E-04	2.212	12
AB002331	Death associated transcription factor 1	DATF1	3.000	1.585	0.713	0.438	7.822	6.12E-05	5.173	10
AK025307	Carnitine palmitoyltransferase I	CPT1	2.996	1.583	0.942	0.595	6.470	3.04E-04	2.890	12

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_018416	FOXJ2 forkhead factor	FHX	2.990	1.580	1.145	0.696	5.326	1.45E-03	0.977	8
NM_006435	Interferon induced transmembrane protein	IFITM2	2.986	1.578	0.943	0.603	6.210	5.55E-04	2.235	10
AL117595	Homo sapiens mRNA		2.981	1.576	0.527	0.417	9.420	2.83E-05	6.414	9
AF064839	Hypothetical protein		2.981	1.576	1.120	0.731	4.978	3.33E-03	-0.028	8
NM_032227	Hypothetical protein FLJ22679	FLJ22679	2.973	1.572	0.973	0.617	6.260	4.01E-04	2.557	11
NM_018030	Oxysterol binding protein-like 1A	OSBPL1A	2.973	1.572	0.588	0.423	6.788	2.05E-04	3.502	9
NM_003440	Zinc finger protein 140 (clone pHZ-39)	ZNF140	2.965	1.568	0.719	0.601	4.385	5.58E-03	-0.966	10
AK055427	Homo sapiens cDNA FLJ30865 fis		2.961	1.566	0.977	0.617	6.076	5.14E-04	2.306	9
NM_000454	Superoxide dismutase 1	SOD1	2.944	1.558	0.324	0.226	10.870	2.89E-06	9.300	11
AK023371	Homo sapiens cDNA FLJ11872 fis		2.932	1.552	0.672	0.510	6.080	5.12E-04	2.238	10
NM_024928	Hypothetical protein FLJ22559	FLJ22559	2.928	1.550	0.956	0.761	4.413	5.38E-03	-0.946	11
NM_006085	3'(2'), 5'-bisphosphate nucleotidase 1	BPNT1	2.910	1.541	1.308	0.827	4.699	3.54E-03	-0.223	8
AK055515	Homo sapiens cDNA: FLJ21260 fis		2.906	1.539	0.862	0.670	4.971	2.37E-03	0.142	11
NM_012417	Retinal degeneration B beta	PITPNC1	2.898	1.535	0.774	0.572	6.100	4.99E-04	2.240	11
BC000073	Hypothetical protein MGC3260	KIAA1271	2.876	1.524	0.964	0.631	5.925	8.05E-04	1.752	10
NM_016101	Hypothetical protein	CGI-37	2.858	1.515	0.968	0.634	5.838	8.96E-04	1.602	10
AK022134	Homo sapiens cDNA FLJ12072 fis	CAT	2.834	1.503	0.552	0.314	6.739	2.17E-04	3.457	8
NM_006457	PDZ and LIM domain 5, transcript variant 1	PDLIM5	2.830	1.501	0.452	0.301	11.960	1.20E-06	10.563	12
BC009838	Homo sapiens	ELOVL5	2.826	1.499	0.842	0.562	6.817	1.98E-04	3.484	12
BC016858	Homo sapiens		2.823	1.497	0.396	0.262	12.940	6.07E-07	11.656	10
NM_020988	Guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O	GNAO1	2.793	1.482	1.095	0.730	5.264	1.58E-03	0.776	10
NM_004841	RAS protein activator like 2	RASAL2	2.793	1.482	0.570	0.374	8.853	1.98E-05	6.733	9
AL133064	Homo sapiens mRNA		2.766	1.468	0.578	0.448	6.346	3.58E-04	2.782	8
Y11339	GalNAc alpha-2	ST6GalNAcI	2.745	1.457	0.941	0.697	6.112	6.31E-04	2.198	8

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
AB046845	E3 ubiquitin ligase SMURF1	SMURF1	2.738	1.453	0.417	0.286	12.200	1.00E-06	10.870	10
AL512749	Homo sapiens mRNA	SLC7A2	2.723	1.445	1.160	0.780	4.823	2.96E-03	-0.028	9
AK022049	Homo sapiens cDNA FLJ11987 fis		2.717	1.442	0.845	0.490	4.923	2.54E-03	0.125	9
NM_024588	Hypothetical protein FLJ23584	FLJ23584	2.715	1.441	0.479	0.334	10.780	3.13E-06	9.214	11
NM_017923	Hypothetical protein FLJ20668	FLJ20668	2.713	1.440	0.520	0.357	9.632	1.44E-05	7.246	9
NM_032188	Histone acetyltransferase MYST1	MYST1	2.698	1.432	1.035	0.718	5.394	1.33E-03	1.020	10
NM_018142	Hypothetical protein FLJ10569	FLJ10569	2.691	1.428	0.748	0.513	6.905	2.39E-04	3.446	8
AK022272	Homo sapiens cDNA FLJ12210 fis		2.683	1.424	0.663	0.521	6.634	2.48E-04	3.173	11
NM_012175	F-box only protein 3	FBXO3	2.663	1.413	0.873	0.751	4.642	3.86E-03	-0.422	9
NM_023948	Hypothetical protein AF053356_CDS3	AF053356_CDS3	2.661	1.412	1.217	0.869	4.544	4.44E-03	-0.614	10
AK001133	Homo sapiens cDNA FLJ10271 fis		2.659	1.411	0.606	0.419	7.842	5.99E-05	5.238	9
NM_003819	Poly(A) binding protein	PABPC4	2.657	1.410	0.931	0.682	6.106	4.95E-04	2.284	22
NM_004964	Histone deacetylase 1	HDAC1	2.656	1.409	0.473	0.363	8.536	2.76E-05	6.215	10
NM_024308	Hypothetical protein MGC4172	MGC4172	2.637	1.399	0.728	0.516	7.317	1.08E-04	4.375	10
NM_004997	Myosin binding protein H	MYBPH	2.634	1.397	0.561	0.458	6.804	2.01E-04	3.495	10
NM_005506	CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 2	CD36L2	2.617	1.388	0.866	0.623	6.136	4.75E-04	2.374	10
NM_032332	Hypothetical protein MGC4238	MGC4238	2.608	1.383	0.742	0.542	6.795	2.03E-04	3.592	8
NM_006730	Deoxyribonuclease I-like 1	DNASE1L1	2.603	1.380	1.058	0.757	5.076	2.04E-03	0.418	10
AL080186	DKFZP564B0769 protein	DKFZp564B0769	2.595	1.376	0.946	0.682	5.761	7.99E-04	1.782	8
AB011123	KIAA0551 protein	KIAA0551	2.590	1.373	0.682	0.474	8.270	3.75E-05	5.822	19
AL049254	Homo sapiens mRNA		2.588	1.372	0.965	0.703	5.476	1.18E-03	1.101	12
NM_003375	Voltage-dependent anion channel 2	VDAC2	2.586	1.371	0.694	0.627	4.973	2.37E-03	0.182	10
NM_004334	Bone marrow stromal cell antigen 1	BST1	2.583	1.369	0.391	0.301	12.000	1.17E-06	10.610	23
NM_014674	KIAA0212 gene product	EDEM1	2.583	1.369	0.995	0.735	5.420	1.28E-03	1.110	9

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_032785	Hypothetical protein FLJ14442		2.511	1.328	0.665	0.489	7.114	1.39E-04	4.044	10
NM_018195	Hypothetical protein FLJ10726	FLJ10726	2.488	1.315	0.575	0.437	8.530	2.77E-05	6.206	11
NM_015972	RNA polymerase I 16 kDa subunit	MGC9850	2.481	1.311	0.344	0.257	10.620	3.56E-06	9.042	10
NM_021073	Bone morphogenetic protein 5	BMP5	2.466	1.302	0.625	0.471	7.448	9.24E-05	4.586	10
AL117638	Homo sapiens mRNA		2.459	1.298	0.625	0.476	7.622	7.62E-05	4.827	11
NM_031475	Espin	ESPN	2.457	1.297	0.902	0.695	5.526	1.10E-03	1.193	12
NM_005300	G protein-coupled receptor 34	GPR34	2.449	1.292	0.768	0.621	6.423	3.24E-04	2.878	20
NM_020529	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	NFKBIA	2.449	1.292	0.774	0.589	6.382	3.40E-04	2.845	9
AK021840	Homo sapiens cDNA FLJ11778 fis		2.447	1.291	0.627	0.486	7.720	6.82E-05	4.951	12
AK000271	Homo sapiens cDNA FLJ20264 fis		2.444	1.289	0.494	0.451	5.667	9.03E-04	1.527	9
NM_006037	Histone deacetylase 4	HDAC4	2.440	1.287	0.799	0.621	6.152	4.65E-04	2.333	12
NM_016084	RAS	RASD1	2.439	1.286	0.495	0.423	7.514	8.57E-05	4.657	10
NM_031431	Tethering factor SEC34	SEC34	2.437	1.285	0.959	0.734	5.151	1.85E-03	0.560	10
NM_032130	Hypothetical protein DKFZp434J0113	DKFZP434J0113	2.435	1.284	0.462	0.296	5.390	1.33E-03	1.052	8
NM_025080	Hypothetical protein FLJ22316	ASRGL1	2.427	1.279	0.735	0.576	6.641	2.46E-04	3.218	11
NM_002633	Phosphoglucomutase 1	PGM1	2.427	1.279	0.527	0.467	6.605	2.56E-04	3.157	10
BC009649	Serologically defined colon cancer antigen 16	SDCCAG16	2.427	1.279	0.357	0.320	7.831	6.07E-05	5.187	9
NM_017892	Formin binding protein 3	FNBP3	2.405	1.266	0.871	0.686	5.604	9.92E-04	1.373	11
NM_005195	CCAAT/enhancer binding protein (C/EBP)	KIAA0146	2.402	1.264	1.137	0.899	4.343	5.96E-03	-1.050	11
AB067506	KIAA1919 protein	NaGLT1	2.402	1.264	1.039	0.825	4.734	3.35E-03	-0.281	11
AJ237663	Homo sapiens ORF1		2.398	1.262	0.790	0.624	6.165	4.58E-04	2.426	10
NM_013975	Ligase III	LIG3	2.393	1.259	0.586	0.520	9.111	2.36E-05	6.646	8
NM_006873	Stoned B/TFIIA-alpha/beta-like factor	SBLF	2.385	1.254	1.032	0.811	4.822	2.96E-03	0.015	8
NM_002971	Special AT-rich sequence binding	SATB1	2.378	1.250	0.599	0.546	6.085	5.08E-04	2.247	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
	protein									
BC008613	Homo sapiens		2.367	1.243	0.474	0.427	6.845	1.92E-04	3.563	10
NM_007007	Cleavage and polyadenylation specific factor 6, 68kDa	CPSF6	2.354	1.235	0.587	0.538	6.284	3.88E-04	2.600	10
NM_004869	Suppressor of K transport defect 1	VPS4B	2.341	1.227	0.612	0.499	7.502	8.71E-05	4.606	12
NM_003930	Src family associated phosphoprotein 2	SCAP2	2.338	1.225	0.998	0.818	4.798	3.07E-03	-0.118	10
NM_015507	EGF-like-domain	EGFL6	2.321	1.215	0.382	0.370	6.854	1.89E-04	3.651	8
AL049390	Homo sapiens mRNA		2.310	1.208	0.787	0.641	5.845	7.09E-04	1.891	9
NM_004582	Rab geranylgeranyltransferase	RABGGTB	2.297	1.200	0.890	0.734	5.070	2.45E-03	0.318	8
BC012851	Uroplakin 1B	UPK1B	2.291	1.196	1.008	0.834	4.677	3.66E-03	-0.266	8
NM_017620	Hypothetical protein FLJ20011		2.288	1.194	0.573	0.470	7.443	9.30E-05	4.577	10
NM_002937	Ribonuclease	RNASE4	2.277	1.187	1.011	0.815	4.560	4.33E-03	-0.494	8
NM_025034	Hypothetical protein FLJ21290	USH1C	2.274	1.185	0.409	0.418	5.952	6.11E-04	2.045	9
AB046784	KIAA1564 protein	HELSNF1	2.261	1.177	0.873	0.722	5.186	1.75E-03	0.712	8
NM_006534	Nuclear receptor coactivator 3	NCOA3	2.253	1.172	0.990	0.838	4.618	3.99E-03	-0.468	10
NM_032865	Hypothetical protein FLJ14950	CTEN	2.249	1.169	0.649	0.712	4.333	6.05E-03	-1.070	10
NM_012208	Histidyl-tRNA synthetase-like	HARSL	2.235	1.160	0.409	0.319	7.595	7.83E-05	4.786	10
NM_031229	Chromosome 20 open reading frame 18	C20orf18	2.228	1.156	0.511	0.400	6.727	2.20E-04	3.332	11
NM_014211	Gamma-aminobutyric acid (GABA) A receptor, pi	GABRP	2.228	1.156	0.926	0.801	4.799	3.06E-03	-0.189	12
NM_015344	Leptin receptor overlapping transcript-like 1	LEPROTL1	2.225	1.154	1.078	0.914	4.143	7.96E-03	-1.407	10
NM_018428	Hepatocellular carcinoma-associated antigen 66	HCA66	2.210	1.144	0.666	0.544	6.611	2.53E-04	3.134	23
BC015994	Homo sapiens		2.192	1.132	0.643	0.568	6.616	2.52E-04	3.142	12
AB037771	KIAA1350 protein		2.192	1.132	0.676	0.597	6.320	3.70E-04	2.629	12
NM_005875	Translation factor sui1 homolog	GC20	2.187	1.129	0.552	0.489	7.564	8.07E-05	4.706	12

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_000962	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	PTGS1	2.184	1.127	0.986	0.865	4.393	5.53E-03	-0.950	11
J05582	Mucin 1, human pancreatic mucin mRNA	MUC1	2.164	1.114	0.770	0.691	5.512	1.12E-03	1.169	12
AK057446	Homo sapiens cDNA FLJ32884 fis		2.161	1.112	0.563	0.509	7.347	1.04E-04	4.498	8
NM_002826	Quiescin Q6	QSCN6	2.157	1.109	0.413	0.372	9.517	1.03E-05	7.565	12
AK002107	Homo sapiens cDNA FLJ11245 fis	RAB3B	2.154	1.107	0.823	0.753	5.138	1.88E-03	0.536	10
NM_032827	Hypothetical protein FLJ14708	HATH6	2.142	1.099	0.896	0.796	4.652	3.81E-03	-0.403	10
NM_019000	Hypothetical protein	FLJ20152	2.139	1.097	0.979	0.860	4.289	6.45E-03	-1.074	9
NM_005746	Pre-B-cell colony-enhancing factor	PBEF	2.132	1.092	0.552	0.504	7.324	1.08E-04	4.353	11
BC004501	Homo sapiens	MGC33867	2.130	1.091	0.557	0.511	7.301	1.10E-04	4.349	10
AK055768	Homo sapiens cDNA FLJ31206 fis		2.130	1.091	1.031	0.938	4.137	8.02E-03	-1.419	10
NM_031959	Keratin associated protein 3.2	KRTAP3-2	2.127	1.089	0.787	0.710	5.195	1.73E-03	0.730	8
AB046843	KIAA1623 protein	KIAA1623	2.111	1.078	0.533	0.405	5.139	1.87E-03	0.580	8
AK024303	Homo sapiens cDNA FLJ14241 fis		2.107	1.075	0.879	0.818	4.692	3.58E-03	-0.398	12
NM_000107	Damage-specific DNA binding protein 2, 48kDa	DDB2	2.104	1.073	0.940	0.888	4.427	5.27E-03	-0.844	10
AF339829	Homo sapiens clone IMAGE:609847		2.104	1.073	0.234	0.201	10.100	5.77E-06	8.362	10
NM_003768	Phosphoprotein enriched in astrocytes 15	PEA15	2.102	1.072	0.663	0.799	4.098	8.50E-03	-1.537	10
NM_004262	Airway trypsin-like protease	HAT	2.092	1.065	0.902	0.858	4.518	4.60E-03	-0.622	9
NM_023078	Hypothetical protein FLJ13852	FLJ13852	2.091	1.064	0.604	0.599	5.485	1.17E-03	1.151	21
NM_016824	Adducin 3 (gamma)	ADD3	2.088	1.062	0.641	0.598	6.164	4.58E-04	2.388	11
NM_000961	Prostaglandin I2 (prostacyclin) synthase	PTGIS	2.088	1.062	0.595	0.560	6.655	2.42E-04	3.209	12
BC012758	Homo sapiens	LOC149603	2.081	1.057	0.875	0.828	4.507	5.37E-03	-0.816	10
NM_004456	Enhancer of zeste homolog 2	EZH2	2.073	1.052	0.896	0.808	4.450	5.10E-03	-0.711	8

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
	(Drosophila)									
NM_003501	Acyl-Coenzyme A oxidase 3	ACOX3	2.071	1.050	0.743	0.888	4.044	9.20E-03	-1.645	10
AF209930	Chordin	CHRD	2.071	1.050	0.862	0.894	4.227	7.08E-03	-1.241	19
NM_003131	Serum response factor (c-fos serum response element-binding transcription factor)	SRF	2.069	1.049	0.597	0.552	6.496	2.93E-04	3.083	8
AK054789	Homo sapiens cDNA FLJ30227 fis		2.069	1.049	0.774	0.732	5.208	1.70E-03	0.668	10
AF130056	Homo sapiens clone FLB5227 PRO1367 mRNA	RNF12	2.068	1.048	0.878	0.806	4.394	5.52E-03	-0.908	10
NM_016410	Hypothetical protein HSPC177	HSPC177	2.056	1.040	0.399	0.384	9.175	1.44E-05	7.097	12
NM_002970	Spermidine/spermine N1-acetyltransferase	SAT	2.053	1.038	0.539	0.614	5.217	1.68E-03	0.613	22
NM_016264	GIOT-2 for gonadotropin inducible transcription repressor-2	ZNF44	2.051	1.036	0.706	0.683	5.608	9.87E-04	1.418	10
NM_052886	Mal	MAL2	2.042	1.030	0.882	0.827	4.538	4.48E-03	-0.538	8
AB067502	KIAA1915 protein	KIAA1915	2.036	1.026	0.697	0.684	5.563	1.05E-03	1.297	11
NM_021622	Pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1	PLEKHA1	2.034	1.024	0.554	0.541	6.841	1.92E-04	3.524	12
X06389	Synaptophysin	SYP	2.032	1.023	0.508	0.486	7.188	1.27E-04	4.203	9
BC013305	Homo sapiens		2.024	1.017	0.744	0.727	5.209	1.70E-03	0.634	11
NM_000343	Solute carrier family 5 (sodium/glucose cotransporter), member 1	SLC5A1	2.021	1.015	0.844	0.969	4.478	5.56E-03	-0.782	9
NM_001356	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked	DDX3X	2.020	1.014	0.449	0.504	6.321	3.69E-04	2.700	9
NM_024836	Hypothetical protein FLJ22301	FLJ22301	2.020	1.014	0.608	0.585	5.862	6.93E-04	1.883	10
NM_002231	Kangai 1 (suppression of tumorigenicity 6, prostate)	KAI1	2.020	1.014	0.933	0.923	4.211	7.22E-03	-1.312	11

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_003250	Thyroid hormone receptor	THRA	2.014	1.010	0.743	0.736	5.169	1.80E-03	0.522	12
NM_053043	Hypothetical protein MGC20460		-2.003	-1.002	0.910	2.407	-4.143	7.96E-03	-1.446	16
BC013164	Homo sapiens cDNA: FLJ22646 fis		-2.006	-1.004	0.953	0.943	-4.114	8.30E-03	-1.466	10
AK024986	Homo sapiens cDNA: FLJ21333 fis	PTEN	-2.010	-1.007	0.399	0.386	-7.372	1.02E-04	4.463	10
AK024302	Homo sapiens cDNA FLJ14240 fis	OCIA	-2.022	-1.016	0.721	0.709	-5.376	1.36E-03	0.948	11
NM_005778	RNA binding motif protein 5	RBM5	-2.027	-1.019	0.967	0.941	-4.066	8.89E-03	-1.600	11
NM_015980	HMP19 protein	HMP19	-2.029	-1.021	0.905	0.883	-4.463	4.99E-03	-0.684	8
NM_023080	Hypothetical protein FLJ20989	FLJ20989	-2.035	-1.025	0.762	1.309	-5.961	6.05E-04	2.025	20
NM_052946	Hypothetical protein MGC20702	NOSTRIN	-2.035	-1.025	0.691	0.664	-5.586	1.01E-03	1.461	8
BC012362	RAP2B, member of RAS oncogene family	RAP2B	-2.039	-1.028	0.682	0.652	-5.648	9.31E-04	1.492	10
NM_000346	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	SOX9	-2.041	-1.029	0.943	0.931	-4.258	6.76E-03	-1.136	9
AK023948	Homo sapiens cDNA FLJ13886 fis		-2.046	-1.033	0.742	0.744	-4.812	3.00E-03	-0.003	8
NM_014635	KIAA0336 gene product	GCC185	-2.051	-1.036	0.890	0.872	-4.445	5.14E-03	-0.767	9
NM_016038	CGI-97 protein	SBDS	-2.078	-1.055	0.802	0.757	-5.048	2.13E-03	0.327	11
NM_004904	cAMP response element-binding protein CRE-Bpa	CREB5	-2.085	-1.060	0.728	0.669	-5.373	1.36E-03	0.980	10
NM_014292	Chromobox homolog 6	CBX6	-2.092	-1.065	0.761	0.708	-5.313	1.47E-03	0.831	11
NM_012071	BUP protein	BUP	-2.097	-1.068	0.623	0.583	-6.431	3.21E-04	2.822	12
NM_022343	17kD fetal brain protein	C9orf19	-2.097	-1.068	0.562	0.517	-6.855	1.89E-04	3.614	10
BC010117	Hypothetical protein MGC13038	BCAN	-2.105	-1.074	0.887	0.818	-4.638	3.88E-03	-0.469	11
NM_001455	Forkhead box O3A	FOXO3A	-2.114	-1.080	0.754	0.827	-4.486	4.84E-03	-0.803	11
NM_006991	Zinc finger protein 197	ZNF197	-2.117	-1.082	0.999	0.980	-4.794	3.60E-03	-0.236	17
NM_021111	Reversion-inducing-cysteine-rich protein	RECK	-2.121	-1.085	0.545	0.626	-8.086	4.58E-05	5.576	18
NM_007043	HIV-1 rev binding protein 2	HRB2	-2.124	-1.087	0.794	0.719	-5.186	1.75E-03	0.627	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_001565	Small inducible cytokine subfamily B (Cys-X-Cys), member 10	CXCL10	-2.144	-1.100	0.363	0.375	-6.962	1.65E-04	3.758	10
BI832330	ESTs		-2.145	-1.101	0.500	0.454	-8.051	4.77E-05	5.461	12
AL110248	Homo sapiens mRNA		-2.145	-1.101	0.694	0.634	-6.003	5.68E-04	2.100	11
NM_001374	Deoxyribonuclease I-like 2	DNASE1L2	-2.160	-1.111	0.354	0.333	-7.080	1.44E-04	4.064	8
NM_021178	Enhancer of invasion 10, Cyclin B1 interacting protein 1, transcript variant 1	CCNB1IP1	-2.161	-1.112	0.619	0.562	-6.630	2.49E-04	3.199	11
BC012882	Homo sapiens	LOC150356	-2.164	-1.114	0.447	0.486	-5.406	1.30E-03	1.004	10
NM_001730	Kruppel-like factor 5 (intestinal)	KLF5	-2.186	-1.128	0.696	0.779	-4.005	9.73E-03	-1.640	8
AL136878	Paraneoplastic cancer-testis-brain antigen	PNMA3	-2.195	-1.134	0.564	0.490	-7.092	1.42E-04	3.974	11
NM_006670	Trophoblast glycoprotein	TPBG	-2.196	-1.135	0.786	0.676	-5.417	1.29E-03	1.062	10
NM_002933	Ribonuclease	RNASE1	-2.198	-1.136	0.843	0.743	-5.192	1.74E-03	0.638	10
NM_003135	Signal recognition particle 19kD	SRP19	-2.198	-1.136	0.590	0.523	-7.120	1.38E-04	4.091	9
NM_022553	SAC2 suppressor of actin mutations 2-like	VPS52	-2.202	-1.139	0.855	0.962	-4.057	9.03E-03	-1.578	17
AK023317	Homo sapiens cDNA FLJ13255 fis	HSPA9B	-2.205	-1.141	0.466	0.357	-6.396	3.35E-04	2.869	8
AL109705	Homo sapiens mRNA full length insert cDNA clone		-2.205	-1.141	0.260	0.229	-13.030	5.83E-07	11.733	11
NM_001498	Glutamate-cysteine ligase	GCLC	-2.210	-1.144	1.090	0.952	-4.091	8.56E-03	-1.550	11
NM_022749	Retinoic acid induced 16	RAI16	-2.211	-1.145	0.952	0.822	-4.655	3.78E-03	-0.395	10
AB023204	Erythrocyte membrane protein band 4.1-like 3	EPB41L3	-2.222	-1.152	0.719	0.652	-5.594	1.00E-03	1.432	9
AK001478	Wnt-1 responsive Cdc42 homolog	WRCH1	-2.230	-1.157	1.008	0.870	-4.462	5.00E-03	-0.814	11
NM_013989	Deiodinase	DIO2	-2.231	-1.158	0.562	0.550	-6.134	4.75E-04	2.300	11
BC000268	Proteasome	PSMB2	-2.255	-1.173	0.699	0.719	-4.650	3.82E-03	-0.445	10
NM_019088	Hypothetical protein F23149_1	PD2	-2.256	-1.174	0.572	0.464	-6.291	3.84E-04	2.687	9

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_002491	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa	NDUFB3	-2.259	-1.176	0.902	0.767	-5.015	2.23E-03	0.227	12
AL136924	RAB5 interacting protein 2	RIN2	-2.261	-1.177	0.724	0.608	-6.094	5.02E-04	2.263	11
BC005847	Hypothetical protein DKFZp667O116		-2.277	-1.187	0.620	0.604	-5.674	8.96E-04	1.502	10
NM_002351	SH2 domain protein 1A	SH2D1A	-2.283	-1.191	1.118	0.928	-4.211	7.22E-03	-1.229	9
AL359931	Ras protein-specfic guanine nucleotide-releasing factor 1	RASGRF1	-2.286	-1.193	0.485	0.348	-6.008	7.23E-04	1.933	8
AK057176	Hypothetical protein 669		-2.288	-1.194	0.990	0.822	-4.686	3.61E-03	-0.336	10
AY027862	Upregulated in colorectal cancer gene 1	UCC1	-2.291	-1.196	0.417	0.344	-9.502	1.04E-05	7.571	11
NM_015544	DKFZP564K1964 protein	DKFZP564K1964	-2.302	-1.203	0.745	0.616	-5.937	7.96E-04	1.771	10
NM_031461	CocoaCrisp	CRISPLD1	-2.317	-1.212	0.931	0.764	-5.136	1.88E-03	0.618	8
NM_020397	CamKI-like protein kinase	CKLiK	-2.320	-1.214	0.357	0.270	-8.721	2.29E-05	6.454	11
AK023942	Homo sapiens cDNA FLJ13880 fis		-2.320	-1.214	0.739	0.609	-6.281	3.90E-04	2.629	10
NM_006667	Progesterone receptor membrane component	PGRMC1	-2.325	-1.217	0.844	0.694	-5.522	1.11E-03	1.186	12
NM_022159	EGF-TM7-latrophilin-related protein	ELTD1	-2.329	-1.220	0.916	0.739	-4.974	2.80E-03	0.097	9
NM_001375	Deoxyribonuclease II	DNASE2	-2.336	-1.224	0.524	0.490	-6.339	3.60E-04	2.663	11
NM_014062	ART-4 protein	NOB1P	-2.338	-1.225	0.941	0.740	-4.762	3.22E-03	-0.146	9
NM_017908	Hypothetical protein FLJ20626		-2.338	-1.225	1.001	0.818	-4.833	2.91E-03	-0.009	9
AK025326	Hypothetical protein FLJ21673		-2.341	-1.227	0.935	0.755	-5.149	1.85E-03	0.599	9
NM_015906	Tripartite motif-containing 33	TRIM33	-2.349	-1.232	0.537	0.374	-5.392	1.33E-03	0.944	11
AB058693	Hypothetical protein FLJ23119		-2.355	-1.236	0.522	0.528	-5.021	2.21E-03	0.274	10
AK025007	Homo sapiens cDNA: FLJ21354 fis	FLJ38771	-2.357	-1.237	1.073	0.860	-4.519	4.60E-03	-0.663	10
BC008191	Pleckstrin homology, Sec7 and coiled-coil domains 3	PSCD3	-2.365	-1.242	1.001	0.818	-4.760	3.23E-03	-0.105	8
NM_015400	DKFZP586N0721 protein	DKFZP586N0721	-2.370	-1.245	0.519	0.536	-4.482	4.86E-03	-0.811	11

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
AK026883	Homo sapiens cDNA: FLJ23230 fis		-2.373	-1.247	0.969	1.392	-6.705	2.26E-04	3.362	16
NM_004425	Extracellular matrix protein 1	ECM1	-2.375	-1.248	0.765	0.503	-4.524	4.57E-03	-0.654	9
NM_009587	Lectin	LGALS9	-2.378	-1.250	0.759	0.600	-6.172	4.54E-04	2.401	11
NM_031437	Hypothetical protein MGC10823	RASSF5	-2.378	-1.250	0.692	0.556	-6.749	2.15E-04	3.515	8
AL122043	Hypothetical protein DKFZp566G1424	C20orf112	-2.390	-1.257	0.560	0.443	-8.340	3.46E-05	5.958	10
BC013082	Seven in absentia homolog 2 (Drosophila)	SIAH2	-2.398	-1.262	0.501	0.362	-6.414	3.28E-04	2.826	10
NM_016048	CGI-111 protein	CGI-111	-2.400	-1.263	0.724	0.571	-6.665	2.39E-04	3.332	9
NM_020401	Nuclear pore complex protein	NUP107	-2.407	-1.267	0.953	0.752	-5.120	1.92E-03	0.429	12
NM_000358	Transforming growth factor	TGFBI	-2.410	-1.269	0.784	0.638	-5.541	1.08E-03	1.334	9
NM_004566	6-phosphofructo-2-kinase/fructose-2	PFKFB3	-2.415	-1.272	1.041	0.826	-4.724	3.41E-03	-0.300	11
NM_017875	Hypothetical protein FLJ20551	FLJ20551	-2.422	-1.276	1.175	0.892	-4.117	8.25E-03	-1.459	10
NM_024960	Chromosome 20 open reading frame 48	PANK2	-2.439	-1.286	0.753	0.586	-6.501	2.91E-04	2.944	12
AF417165	5'-nucleotidase, cytosolic IB	NT5C1B	-2.444	-1.289	0.857	0.656	-5.770	7.91E-04	1.715	10
AK024911	Heterogeneous nuclear ribonucleoprotein	HNRPM	-2.452	-1.294	0.981	0.757	-5.118	1.93E-03	0.460	11
NM_002689	Polymerase (DNA-directed)	POLA2	-2.452	-1.294	0.778	0.575	-5.943	6.18E-04	2.068	9
NM_003426	Zinc finger protein 74 (Cos52)	ZNF74	-2.455	-1.296	0.852	0.644	-5.725	8.37E-04	1.634	10
NM_013312	Hook2 protein	HOOK2	-2.462	-1.300	0.950	0.601	-5.541	1.08E-03	1.222	22
NM_032640	Hypothetical protein MGC10526	MGC10526	-2.467	-1.303	0.988	0.778	-4.866	2.76E-03	0.015	10
NM_003054	Solute carrier family 18 (vesicular monoamine), member 2	SLC18A2	-2.467	-1.303	0.677	0.422	-4.780	3.13E-03	-0.110	8
AK024192	Homo sapiens cDNA FLJ14130 fis		-2.469	-1.304	0.727	0.558	-6.841	1.92E-04	3.557	11
AK021643	Homo sapiens cDNA FLJ11581 fis		-2.471	-1.305	0.533	0.411	-8.951	1.82E-05	6.810	11
NM_001788	CDC10 cell division cycle 10 homolog (S. cerevisiae)	CDC10	-2.481	-1.311	0.823	0.809	-4.154	8.72E-03	-1.446	8
NM_052859	Putative endoplasmic reticulum multispan transmembrane protein	RFT1	-2.491	-1.317	0.494	0.375	-9.755	8.21E-06	7.936	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_032626	Retinoblastoma binding protein 6, transcript variant 3	RBBP6	-2.497	-1.320	0.764	1.099	-4.539	4.47E-03	-0.663	20
NM_006007	Zinc finger protein 216	ZNF216	-2.500	-1.322	0.861	0.637	-5.764	7.97E-04	1.743	9
AF029729	Neuralized-like (Drosophila)	NEURL	-2.507	-1.326	1.110	0.839	-4.661	3.75E-03	-0.423	11
AK057278	Contactin 4	CNTN4	-2.519	-1.333	0.834	0.629	-6.025	5.52E-04	2.215	9
NM_002447	Macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	MST1R	-2.521	-1.334	1.110	0.819	-4.744	3.31E-03	-0.181	9
NM_012080	DNA segment	FAM16AX	-2.535	-1.342	1.218	0.868	-4.216	7.19E-03	-1.220	9
NM_032982	Caspase 2	CASP2	-2.537	-1.343	0.596	0.438	-7.554	8.14E-05	4.827	8
AJ000503	Dopachrome tautomerase (dopachrome delta-isomerase, tyrosine-related protein 2)	DCT	-2.558	-1.355	1.114	0.807	-4.701	3.53E-03	-0.307	10
AK024127	Homo sapiens cDNA FLJ14065 fis	SNRP70	-2.558	-1.355	1.090	0.825	-4.421	5.33E-03	-0.768	8
NM_001664	Ras homolog gene family	ARHA	-2.562	-1.357	0.803	0.709	-4.814	2.99E-03	-0.161	11
NM_013380	Zinc finger protein 228	ZNF228	-2.562	-1.357	0.957	0.720	-5.055	2.50E-03	0.292	8
U14383	Mucin 8	MUC8	-2.578	-1.366	0.248	0.193	-12.640	7.30E-07	11.358	8
L19267	Dystrophia myotonica-containing WD repeat motif	DMWD	-2.588	-1.372	0.932	0.681	-5.696	8.69E-04	1.542	11
NM_024954	Hypothetical protein FLJ11807	FLJ11807	-2.597	-1.377	0.250	0.171	-12.620	7.30E-07	11.287	11
NM_002655	Pleiomorphic adenoma gene 1	PLAG1	-2.599	-1.378	0.967	0.702	-5.491	1.16E-03	1.129	12
NM_003809	Tumor necrosis factor (ligand) superfamily, member 12	TNFSF12	-2.604	-1.381	0.450	0.325	-11.000	2.56E-06	9.476	11
NM_005582	Lymphocyte antigen 64 homolog	LY64	-2.606	-1.382	1.008	0.751	-4.908	2.60E-03	0.056	11
BC011808	Chromosome 1 open reading frame 28		-2.606	-1.382	1.083	0.784	-4.939	2.49E-03	0.081	12
NM_022154	Up-regulated by BCG-CWS	BIGM103	-2.608	-1.383	0.545	0.394	-9.372	1.19E-05	7.367	12
NM_024640	Hypothetical protein FLJ23476	FLJ23476	-2.610	-1.384	0.442	0.357	-8.036	4.86E-05	5.535	8
NM_014371	Neighbor of A-kinase anchoring protein 9	NAKAP95	-2.621	-1.390	0.551	0.461	-6.212	4.29E-04	2.438	11
NM_014766	KIAA0193 gene product	KIAA0193	-2.624	-1.392	0.627	0.429	-7.496	8.74E-05	4.736	8

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
AB058773	KIAA1870 protein	COL27A1	-2.630	-1.395	0.573	0.395	-7.562	8.08E-05	4.801	9
NM_030578	Hypothetical protein MGC4093	MGC4093	-2.643	-1.402	0.767	0.555	-6.752	2.14E-04	3.479	16
AK021427	Homo sapiens cDNA FLJ11365 fis		-2.657	-1.410	1.153	0.801	-4.699	3.54E-03	-0.311	10
AK057456	Homo sapiens cDNA FLJ32894 fis		-2.657	-1.410	1.259	0.899	-4.407	5.42E-03	-0.883	10
AK025706	Adenosine monophosphate deaminase 2 (isoform L)	AMPD2	-2.659	-1.411	0.492	0.351	-9.431	1.13E-05	7.503	10
NM_031426	Hypothetical protein FLJ12783	IBA2	-2.661	-1.412	0.706	0.500	-7.547	8.20E-05	4.743	10
BC017907	Homo sapiens		-2.665	-1.414	0.784	0.745	-5.622	9.66E-04	1.407	21
AK001279	Homo sapiens cDNA FLJ10417 fis		-2.674	-1.419	0.694	0.489	-7.731	6.74E-05	4.968	12
AK055877	Homo sapiens cDNA FLJ31315 fis		-2.678	-1.421	0.640	0.537	-5.365	1.38E-03	0.928	10
NM_003684	MAP kinase-interacting serine/threonine	MKNK1	-2.689	-1.427	0.725	0.503	-7.361	1.03E-04	4.412	11
NM_001150	Alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	ANPEP	-2.693	-1.429	1.324	0.915	-4.294	6.40E-03	-1.064	9
NM_024770	Hypothetical protein FLJ13984	FLJ13984	-2.696	-1.431	0.489	0.331	-9.710	8.62E-06	7.935	8
NM_002855	Poliovirus receptor-related 1 (herpesvirus entry mediator C; nectin)	PVRL1	-2.700	-1.433	0.880	0.582	-5.895	6.64E-04	2.024	8
AB058755	Hypothetical protein FLJ14260		-2.702	-1.434	0.525	0.330	-6.961	1.65E-04	3.828	8
AB023210	KIAA0993 protein		-2.704	-1.435	1.221	0.852	-4.599	4.10E-03	-0.544	11
AK022928	Helicase, lympoid-specific	HELLS	-2.715	-1.441	0.690	0.479	-7.900	5.57E-05	5.231	12
AK055808	Homo sapiens		-2.717	-1.442	0.984	0.679	-5.712	8.51E-04	1.608	10
NM_005573	Lamin B1	LMNB1	-2.721	-1.444	0.833	0.580	-6.706	2.26E-04	3.364	10
AB038651	K562 cell-derived leucine-zipper-like protein 1	KLP1	-2.723	-1.445	1.106	0.975	-4.017	9.57E-03	-1.698	21
AL512731	Homo sapiens mRNA		-2.725	-1.446	1.337	0.915	-4.206	7.27E-03	-1.321	11
AK026717	Selenoprotein W, 1	SEPW1	-2.734	-1.451	1.141	0.786	-4.931	2.51E-03	0.066	12
NM_018222	Parvin	PARVA	-2.738	-1.453	1.422	0.972	-4.011	9.65E-03	-1.628	9

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_013438	Ubiquilin 1	UBQLN1	-2.742	-1.455	0.664	0.448	-7.994	5.07E-05	5.437	10
AK023151	Hypothetical protein FLJ13089		-2.743	-1.456	0.570	0.387	-8.866	2.98E-05	6.226	10
NM_014723	Syntaphilin	SNPH	-2.753	-1.461	0.301	0.226	-8.969	1.80E-05	6.897	8
NM_032816	Hypothetical protein FLJ14640		-2.757	-1.463	0.762	0.532	-6.805	2.01E-04	3.609	8
AK056965	Homo sapiens cDNA FLJ32403 fis	MGC2744	-2.759	-1.464	1.041	0.700	-5.431	1.26E-03	1.090	10
NM_014599	EH-domain containing 4	MAGED2	-2.770	-1.470	1.170	0.796	-4.875	2.73E-03	-0.042	12
AK000757	Homo sapiens cDNA FLJ20750 fis	SORT1	-2.778	-1.474	1.299	0.863	-4.453	5.08E-03	-0.750	9
NM_002160	Hexabrachion (tenascin C	TNC	-2.788	-1.479	0.986	0.645	-5.300	1.50E-03	0.844	10
AF055018	Homo sapiens clone 24442 mRNA sequence		-2.795	-1.483	0.851	0.500	-5.354	1.40E-03	0.906	10
BC013113	Homo sapiens	MGC33212	-2.803	-1.487	0.950	0.624	-5.700	1.08E-03	1.401	9
X95097	Vasoactive intestinal peptide receptor 2	VIPR2	-2.803	-1.487	0.804	0.537	-7.173	1.30E-04	4.216	8
AJ414564	Homo sapiens mRNA for connexin40.1 (CX40.1 gene)		-2.809	-1.490	0.404	0.244	-7.626	7.58E-05	4.902	8
NM_001966	Enoyl-Coenzyme A	EHHADH	-2.832	-1.502	0.552	0.361	-9.806	7.80E-06	8.031	9
BC001043	Hypothetical protein DKFZp434E2216	SF4	-2.840	-1.506	0.750	0.579	-5.611	9.84E-04	1.387	10
NM_024567	Hypothetical protein FLJ21616	FLJ21616	-2.844	-1.508	0.770	0.508	-7.458	9.17E-05	4.567	11
AL359954	TAR DNA binding protein-like	TARDBPL	-2.846	-1.509	1.199	0.787	-4.984	2.33E-03	0.283	9
NM_000900	Matrix Gla protein	MGP	-2.852	-1.512	0.963	0.637	-6.052	5.30E-04	2.154	12
BC002770	Homo sapiens	LOC92154	-2.860	-1.516	0.461	0.283	-9.546	1.02E-05	7.604	11
NM_002268	Karyopherin alpha 4 (importin alpha 3)	KPNA4	-2.864	-1.518	0.919	0.771	-4.553	4.38E-03	-0.597	9
NM_001033	Ribonucleotide reductase M1 polypeptide	RRM1	-2.882	-1.527	0.452	0.298	-11.920	1.24E-06	10.562	10
NM_012142	Cyclin D-type binding-protein 1	CCNDBP1	-2.900	-1.536	0.935	0.604	-6.371	3.45E-04	2.788	10
AK056622	Homo sapiens cDNA FLJ32060 fis	PPHLN1	-2.918	-1.545	1.276	0.816	-4.848	2.84E-03	0.067	8
BC001698	Lipase protein	ABHD6	-2.936	-1.554	0.596	0.380	-9.214	2.14E-05	6.684	10
NM_001558	Interleukin 10 receptor	IL10RA	-2.938	-1.555	0.727	0.470	-7.981	5.13E-05	5.488	8
NM_002378	Megakaryocyte-associated tyrosine	MATK	-2.938	-1.555	1.264	0.816	-4.786	3.11E-03	-0.140	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
	kinase									
AL122055	KIAA1028 protein		-2.951	-1.561	0.854	0.542	-6.927	1.72E-04	3.701	11
NM_000582	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	-2.965	-1.568	1.196	0.922	-4.142	7.98E-03	-1.486	11
NM_000609	Stromal cell-derived factor 1	CXCL12	-2.977	-1.574	1.330	0.830	-4.610	4.03E-03	-0.397	8
NM_001275	Chromogranin A (parathyroid secretory protein 1)	CHGA	-2.998	-1.584	0.564	0.409	-7.324	1.08E-04	4.423	8
AB067491	KIAA1904 protein	KIAA1904	-3.042	-1.605	0.453	0.331	-6.835	1.94E-04	3.581	9
AK022190	Homo sapiens cDNA FLJ12128 fis		-3.059	-1.613	1.095	0.682	-5.728	8.36E-04	1.602	11
AF261091	ATP-binding cassette	ABCF2	-3.067	-1.617	0.599	0.430	-7.036	1.51E-04	3.916	9
NM_004688	N-myc (and STAT) interactor	NMI	-3.072	-1.619	0.872	0.539	-7.119	1.38E-04	3.987	12
AK023345	Homo sapiens cDNA FLJ13283 fis	DIAPH1	-3.089	-1.627	1.042	0.637	-6.055	5.28E-04	2.193	11
NM_020634	Growth differentiation factor 3	GDF3	-3.093	-1.629	1.217	0.733	-5.266	1.58E-03	0.864	8
AK057556	Homo sapiens cDNA FLJ32994 fis		-3.093	-1.629	1.392	0.856	-4.594	4.13E-03	-0.554	11
NM_016022	CGI-78 protein	APH-1A	-3.095	-1.630	0.726	0.544	-5.427	1.26E-03	1.045	10
AK058118	Homo sapiens cDNA FLJ25389 fis		-3.095	-1.630	0.641	0.397	-9.526	1.03E-05	7.690	8
AK056022	Homo sapiens cDNA FLJ31460 fis	UXS1	-3.097	-1.631	1.095	0.667	-5.836	7.19E-04	1.835	10
NM_004772	P311 protein	C5orf13	-3.110	-1.637	0.965	0.589	-6.536	2.79E-04	3.004	12
AK057169	Small acidic protein		-3.110	-1.637	1.100	0.654	-5.862	6.93E-04	1.964	8
AK055600	Popeye protein 3		-3.112	-1.638	0.501	0.307	-11.990	1.18E-06	10.676	8
NM_001618	ADP-ribosyltransferase NAD(+)	ADPRT	-3.117	-1.640	0.494	0.293	-11.110	2.36E-06	9.671	8
NM_006144	Granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	GZMA	-3.134	-1.648	0.750	0.383	-5.504	1.14E-03	1.225	9
AP001753	Homo sapiens genomic DNA		-3.145	-1.653	1.402	0.832	-4.604	4.07E-03	-0.496	10
NM_003910	Maternal G10 transcript	G10	-3.156	-1.658	0.885	0.667	-4.313	6.23E-03	-1.069	9
AF368463	Carboxypeptidase M	CPM	-3.176	-1.667	1.071	0.642	-6.021	5.55E-04	2.098	12
AB026156	Homo sapiens mRNA for TCAM-1		-3.187	-1.672	0.584	0.345	-10.040	6.09E-06	8.285	11

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_003576	Serine/threonine kinase 24 (STE20 homolog, yeast)	STK24	-3.187	-1.672	1.314	0.770	-5.033	2.18E-03	0.262	24
NM_024903	Hypothetical protein FLJ14297	FLJ14297	-3.193	-1.675	0.601	0.352	-10.010	6.28E-06	8.301	9
AF056490	Phosphodiesterase 8A	PDE8A	-3.215	-1.685	1.514	0.885	-4.381	5.60E-03	-0.935	10
AK021648	Homo sapiens cDNA FLJ11586 fis		-3.220	-1.687	0.461	0.252	-9.922	1.14E-05	7.577	9
NM_012261	Chromosome 20 open reading frame 103	C20orf103	-3.224	-1.689	0.770	0.456	-8.145	6.23E-05	5.301	8
L36861	Guanylate cyclase activator 1A (retina)	GUCA1A	-3.229	-1.691	0.931	0.540	-6.951	1.67E-04	3.813	9
NM_000129	Coagulation factor XIII	F13A1	-3.238	-1.695	1.324	0.761	-4.582	4.83E-03	-0.586	8
NM_004651	Ubiquitin specific protease 11	USP11	-3.242	-1.697	0.701	0.442	-7.767	6.52E-05	5.122	18
AK056432	Homo sapiens cDNA FLJ31870 fis		-3.242	-1.697	0.817	0.481	-7.942	5.32E-05	5.296	12
AK021706	Homo sapiens cDNA FLJ11644 fis		-3.242	-1.697	0.362	0.194	-10.140	9.47E-06	7.845	9
NM_002700	POU domain, class 4, transcription factor 3	POU4F3	-3.249	-1.700	1.085	0.628	-6.131	4.77E-04	2.403	9
NM_024741	Hypothetical protein FLJ12827	ZNF408	-3.256	-1.703	1.347	0.781	-4.955	2.43E-03	0.186	10
AB033058	KIAA1232 protein	DLG3	-3.263	-1.706	1.105	0.643	-6.022	5.54E-04	2.171	10
NM_002449	Msh homeo box homolog 2 (Drosophila)	MSX2	-3.276	-1.712	1.517	0.876	-4.393	6.28E-03	-0.944	8
NM_013421	Gamma-glutamyltransferase 1	GGT1	-3.297	-1.721	0.904	0.524	-7.360	1.03E-04	4.410	11
NM_033419	Hypothetical gene MGC9753	CAB2	-3.324	-1.733	1.090	0.621	-6.057	5.27E-04	2.197	11
NM_005748	YY1 associated factor 2	YAF2	-3.326	-1.734	1.507	0.857	-4.513	4.63E-03	-0.586	8
AK021640	Homo sapiens cDNA FLJ11578 fis		-3.336	-1.738	0.378	0.204	-11.710	1.43E-06	10.301	10
NM_016231	Nemo-like kinase	NLK	-3.340	-1.740	1.477	0.838	-4.645	3.84E-03	-0.416	10
NM_003468	Frizzled homolog 5 (Drosophila)	FZD5	-3.366	-1.751	1.716	0.965	-4.083	8.68E-03	-1.438	8
AK025311	Homo sapiens cDNA: FLJ21658 fis		-3.418	-1.773	0.377	0.230	-11.150	2.30E-06	9.677	9
NM_005463	Heterogeneous nuclear ribonucleoprotein	HNRPDL	-3.434	-1.780	0.951	0.674	-6.747	2.15E-04	3.399	20
BC011684	GATA zinc finger domain containing 2A	GATAD2A	-3.439	-1.782	0.989	0.672	-5.193	1.74E-03	0.640	9
AF305616	Transmembrane, prostate androgen induced RNA	TMEPAI	-3.465	-1.793	0.624	0.345	-10.400	4.28E-06	8.745	11

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_021197	WAP four-disulfide core domain 1	WFDC1	-3.468	-1.794	1.064	0.576	-5.961	6.05E-04	2.100	9
Z98751	Human DNA sequence from PAC 560B9 on chromosome 1q24-25		-3.468	-1.794	0.927	0.517	-7.451	9.23E-05	4.525	12
NM_006447	Ubiquitin specific protease 16	USP16	-3.473	-1.796	1.385	0.769	-5.138	1.88E-03	0.537	10
NM_002541	Oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide)	OGDH	-3.473	-1.796	1.134	0.714	-6.623	2.50E-04	3.222	19
AK054771	Homo sapiens cDNA FLJ30209 fis		-3.482	-1.800	0.500	0.305	-9.307	1.26E-05	7.306	10
NM_007240	Dual specificity phosphatase 12	DUSP12	-3.485	-1.801	1.587	0.862	-4.415	5.37E-03	-0.825	9
NM_002427	Matrix metalloproteinase 13 (collagenase 3)	MMP13	-3.506	-1.810	0.897	0.501	-7.754	6.60E-05	5.103	9
NM_014456	Programmed cell death 4 (neoplastic transformation inhibitor)	PDCD4	-3.506	-1.810	0.442	0.265	-10.520	3.87E-06	8.860	11
AF161383	Homo sapiens	LOC154467	-3.509	-1.811	0.401	0.207	-11.940	1.22E-06	10.561	10
AK023375	Homo sapiens cDNA FLJ13313 fis		-3.521	-1.816	0.353	0.177	-11.080	2.39E-06	9.596	9
NM_030570	Hypothetical protein MGC10902	UPK3B	-3.523	-1.817	0.562	0.307	-11.710	1.43E-06	10.347	9
BC002821	Homo sapiens	CBX1	-3.531	-1.820	1.084	0.588	-6.550	2.74E-04	3.135	9
NM_016816	2',5'-oligoadenylate synthetase 1, 40/46kDa	OAS1	-3.536	-1.822	0.494	0.272	-11.790	1.38E-06	10.455	8
Z84479	RAR (RAS like GTPASE) like	RARL	-3.568	-1.835	0.987	0.579	-7.630	7.56E-05	4.841	20
NM_005954	Metallothionein 3 (growth inhibitory factor (neurotrophic))	MT3	-3.570	-1.836	0.638	0.347	-10.810	3.07E-06	9.227	12
AK022028	Homo sapiens cDNA FLJ11966 fis		-3.578	-1.839	1.015	0.554	-7.167	1.31E-04	4.168	9
NM_018356	Hypothetical protein FLJ11193		-3.585	-1.842	1.717	0.926	-4.261	6.74E-03	-1.173	10
AK027328	Homo sapiens cDNA FLJ14422 fis		-3.585	-1.842	0.421	0.225	-14.630	1.93E-07	13.314	8
NM_000151	Glucose-6-phosphatase, catalytic (glycogen storage disease type I, von Gierke disease)	G6PC	-3.603	-1.849	0.511	0.296	-10.630	3.56E-06	9.023	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_018964	Solute carrier family 37 (glycerol-3-phosphate transporter), member 1	SLC37A1	-3.633	-1.861	0.566	0.273	-8.553	2.73E-05	6.270	9
NM_032349	Hypothetical protein MGC11275	SDOS	-3.635	-1.862	1.508	0.803	-4.920	2.56E-03	0.160	9
AB040893	KIAA1460 protein		-3.635	-1.862	1.068	0.574	-6.747	2.15E-04	3.365	12
BC001255	Nuclear cap binding protein subunit 2	NCBP2	-3.645	-1.866	0.831	0.424	-7.187	1.72E-04	3.886	8
NM_006245	Protein phosphatase 2	PPP2R5D	-3.648	-1.867	0.640	0.379	-8.236	3.90E-05	5.741	11
L40392	S164 protein		-3.673	-1.877	1.381	0.736	-5.296	1.51E-03	0.763	12
AK057628	Homo sapiens cDNA FLJ33066 fis		-3.673	-1.877	1.265	0.670	-5.598	1.24E-03	1.181	10
AK056153	Deleted in esophageal cancer 1	1-Dec	-3.676	-1.878	0.904	0.553	-6.067	5.20E-04	2.215	10
NM_024018	Butyrophilin	BTN2A3	-3.686	-1.882	1.247	0.663	-5.868	6.88E-04	1.822	12
NM_000086	Ceroid-lipofuscinosis	CLN3	-3.704	-1.889	1.680	0.889	-4.395	5.52E-03	-0.983	12
NM_018046	Hypothetical protein FLJ10283	FLJ10283	-3.727	-1.898	0.561	0.295	-12.320	8.99E-07	10.999	10
AK056574	Homo sapiens cDNA FLJ32012 fis		-3.737	-1.902	0.465	0.243	-14.230	2.47E-07	12.933	10
NM_003090	Small nuclear ribonucleoprotein polypeptide A'	SNRPA1	-3.784	-1.920	1.781	0.887	-4.092	8.56E-03	-1.420	8
AB051461	KIAA1674	KIAA1674	-3.789	-1.922	1.167	0.610	-6.416	3.27E-04	2.830	11
NM_052818	Hypothetical gene CG018	CG018	-3.803	-1.927	1.821	0.941	-4.260	6.75E-03	-1.133	9
AK021495	Homo sapiens cDNA FLJ11433 fis		-3.805	-1.928	0.414	0.199	-11.590	1.57E-06	10.164	10
AK023870	Homo sapiens cDNA FLJ13808 fis		-3.837	-1.940	0.914	0.471	-8.082	6.62E-05	5.212	8
AL136752	Hypothetical protein FLJ12716	FLJ12716	-3.842	-1.942	1.478	0.763	-4.879	3.82E-03	-0.203	8
AK022035	Homo sapiens cDNA FLJ11973 fis		-3.853	-1.946	0.888	0.531	-5.850	7.05E-04	1.824	10
AK057607	Homo sapiens cDNA FLJ33045 fis		-3.907	-1.966	0.980	0.484	-7.728	6.76E-05	5.100	8
NM_015701	Homo sapiens chromosome 2 open reading frame 30	C2orf30	-3.931	-1.975	1.373	0.860	-4.211	7.22E-03	-1.311	10
AJ420564	Homo sapiens cDNA: FLJ22314 fis		-3.950	-1.982	1.141	0.561	-6.513	2.86E-04	3.072	9
NM_019110	Hypothetical protein P1 p373c6	P1P373C6	-3.967	-1.988	0.592	0.276	-9.958	6.67E-06	8.175	10
NM_033105	Beta cysteine string protein	CSP-beta	-3.975	-1.991	1.470	0.738	-5.364	1.38E-03	0.964	10
NM_003648	Diacylglycerol kinase	DGKD	-3.981	-1.993	1.288	0.631	-5.868	6.88E-04	1.894	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
AL137340	Hypothetical protein DKFZp761C1711		-3.986	-1.995	0.723	0.360	-10.500	3.92E-06	8.914	9
AK023330	Homo sapiens cDNA FLJ13268 fis		-3.989	-1.996	0.278	0.139	-11.070	2.40E-06	9.556	9
NM_032013	NDRG family member 3	NDRG3	-3.997	-1.999	1.630	0.802	-4.821	2.96E-03	-0.072	10
NM_012119	Cell cycle related kinase	CCRK	-4.017	-2.006	1.474	0.741	-5.309	1.48E-03	0.823	11
AK058080	Homo sapiens cDNA FLJ25351 fis		-4.025	-2.009	0.951	0.541	-6.199	4.37E-04	2.485	9
NM_005566	Lactate dehydrogenase A	LDHA	-4.081	-2.029	0.690	4.312	-14.710	1.93E-07	13.390	142
BC017595	Homo sapiens		-4.124	-2.044	1.700	0.820	-4.795	3.08E-03	-0.082	9
AK021636	Homo sapiens cDNA FLJ11574 fis	C14orf111	-4.141	-2.050	0.240	0.111	-15.710	1.04E-07	14.265	8
NM_005741	Zinc finger protein 263	ZNF263	-4.161	-2.057	1.472	0.717	-5.490	1.16E-03	1.161	11
NM_002557	Oviductal glycoprotein 1	OVGP1	-4.190	-2.067	1.563	0.761	-5.223	1.67E-03	0.697	10
NM_032307	Hypothetical protein MGC10999	MGC10999	-4.208	-2.073	0.944	0.518	-6.731	2.20E-04	3.370	10
AK021524	Homo sapiens cDNA FLJ11462 fis	PRPF18	-4.208	-2.073	0.429	0.190	-11.100	2.36E-06	9.590	10
AK023300	Homo sapiens cDNA FLJ13238 fis		-4.225	-2.079	0.381	0.171	-12.840	6.28E-07	11.547	9
AL157773	Human DNA sequence from clone RP11-345L2		-4.234	-2.082	0.801	0.385	-9.643	1.44E-05	7.231	10
NM_025005	Hypothetical protein FLJ13315	FLJ13315	-4.243	-2.085	0.251	0.113	-15.040	1.53E-07	13.680	8
AK054573	Homo sapiens cDNA FLJ30011 fis		-4.272	-2.095	1.406	0.668	-5.719	8.43E-04	1.704	8
AF350881	Transient receptor potential cation channel, subfamily M, member 6	TRPM6	-4.293	-2.102	0.841	0.363	-7.139	1.35E-04	4.052	10
AK023112	Homo sapiens cDNA FLJ13050 fis		-4.359	-2.124	1.162	0.545	-7.158	1.32E-04	4.117	10
AF130085	Homo sapiens clone FLB8503 PRO2286 mRNA	CTNNB1	-4.365	-2.126	0.759	0.356	-10.650	3.48E-06	9.054	11
AK056459	Hypothetical protein FLJ22938		-4.374	-2.129	1.332	0.620	-6.205	4.33E-04	2.461	11
NM_015898	HIV-1 inducer of short transcripts binding protein	FBI1	-4.411	-2.141	1.176	0.540	-6.943	1.69E-04	3.761	10
AK023058	Homo sapiens cDNA FLJ12996 fis		-4.411	-2.141	1.037	0.497	-6.855	2.51E-04	3.290	10
NM_014882	KIAA0053 gene product	KIAA0053	-4.438	-2.150	0.660	0.303	-11.750	1.42E-06	10.371	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_004434	Echinoderm microtubule-associated protein like 1	EML1	-4.451	-2.154	1.024	0.471	-7.953	5.25E-05	5.409	9
NM_004237	Thyroid hormone receptor interactor 13	TRIP13	-4.466	-2.159	0.623	0.287	-12.850	6.23E-07	11.581	9
NM_012341	G protein-binding protein CRFG	GTPBP4	-4.494	-2.168	0.500	0.204	-8.141	4.32E-05	5.628	10
AK022859	Homo sapiens cDNA FLJ12797 fis	UNC5B	-4.506	-2.172	1.810	0.819	-4.779	3.14E-03	-0.113	9
NM_018294	Hypothetical protein FLJ10998	FLJ10998	-4.513	-2.174	0.707	0.388	-6.720	2.23E-04	3.425	8
NM_017597	Hypothetical protein DKFZp761K1824	AMSH-LP	-4.528	-2.179	1.512	0.687	-5.604	9.92E-04	1.373	11
NM_032315	Hypothetical protein MGC4399	MGC4399	-4.538	-2.182	1.668	0.762	-4.989	2.73E-03	0.085	10
AB020626	KIAA0819 protein	KIAA0819	-4.547	-2.185	0.658	0.298	-11.820	1.37E-06	10.426	11
NM_007117	Thyrotropin-releasing hormone	TRH	-4.560	-2.189	1.118	0.506	-7.509	8.63E-05	4.649	11
NM_005432	X-ray repair complementing defective repair in Chinese hamster cells 3	XRCC3	-4.582	-2.196	1.388	0.630	-6.100	4.99E-04	2.390	8
NM_024999	Hypothetical protein FLJ12988	FLJ12988	-4.611	-2.205	0.708	0.321	-11.790	1.38E-06	10.379	12
BC011941	Homo sapiens		-4.624	-2.209	0.831	0.316	-5.161	1.82E-03	0.542	10
AB051448	KIAA1661 protein	KIAA1661	-4.640	-2.214	1.081	0.488	-7.924	5.44E-05	5.268	12
NM_014887	Hypothetical protein from BCRA2 region	CG005	-4.665	-2.222	1.581	0.693	-5.599	9.97E-04	1.486	8
NM_006080	Sema domain	SEMA3A	-4.665	-2.222	0.660	0.294	-12.320	8.99E-07	11.037	8
AK022930	Homo sapiens cDNA FLJ12868 fis	YME1L1	-4.672	-2.224	0.518	0.220	-12.620	7.30E-07	11.288	11
NM_001200	Bone morphogenetic protein 2	BMP2	-4.691	-2.230	1.198	0.632	-5.441	1.24E-03	1.071	10
NM_006518	Small proline-rich protein 2C	SPRR2C	-4.708	-2.235	0.582	0.257	-13.320	4.76E-07	12.045	10
NM_004217	Serine/threonine kinase 12	AURKB	-4.724	-2.240	0.770	0.377	-8.170	4.17E-05	5.671	10
NM_001878	Cellular retinoic acid binding protein 2	CRABP2	-4.740	-2.245	1.757	0.783	-4.997	2.28E-03	0.193	12
AK024419	Homo sapiens mRNA for FLJ00008 protein		-4.744	-2.246	0.765	0.378	-8.170	4.17E-05	5.643	11
BC010642	Zinc finger protein 22 (KOX 15)	ZNF22	-4.763	-2.252	0.837	0.473	-4.813	2.99E-03	-0.127	10
AK000893	Homo sapiens cDNA FLJ10031 fis		-4.800	-2.263	1.421	0.615	-6.262	4.00E-04	2.635	9
NM_025004	Hypothetical protein FLJ13215	FLJ13215	-4.803	-2.264	0.313	0.131	-16.860	5.39E-08	15.214	9

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_006621	S-adenosylhomocysteine hydrolase-like 1	AHCYL1	-4.817	-2.268	1.247	0.545	-6.897	1.79E-04	3.686	10
NM_006749	Solute carrier family 20 (phosphate transporter), member 2	SLC20A2	-4.820	-2.269	1.122	0.494	-7.388	9.96E-05	4.490	10
AL133269	Human DNA sequence from clone RP3-468K3		-4.820	-2.269	1.035	0.450	-8.122	6.39E-05	5.233	9
NM_024810	Hypothetical protein FLJ23018	FLJ23018	-4.847	-2.277	1.237	0.551	-7.097	1.41E-04	4.017	20
AK026809	Homo sapiens cDNA: FLJ23156 fis		-4.911	-2.296	1.798	0.782	-5.035	2.17E-03	0.302	11
AL137398	Homo sapiens mRNA		-4.918	-2.298	1.258	0.692	-5.431	1.26E-03	1.090	17
NM_030964	Sprouty homolog 4 (Drosophila)	SPRY4	-4.952	-2.308	0.446	0.180	-12.940	6.07E-07	11.642	10
AF176707	F-box only protein 29	FBX29	-4.986	-2.318	0.346	0.141	-15.180	1.40E-07	13.794	10
AB040950	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 37	DHX37	-4.990	-2.319	1.187	0.498	-7.419	9.60E-05	4.575	9
NM_024324	Cysteine-rich with EGF-like domains 2	CRELD2	-5.004	-2.323	1.526	0.621	-6.519	2.84E-04	3.008	21
AK055159	Homo sapiens cDNA FLJ30597 fis		-5.004	-2.323	1.725	0.724	-5.279	1.55E-03	0.844	9
AK022094	Homo sapiens cDNA FLJ12032 fis		-5.018	-2.327	0.963	0.400	-8.944	1.83E-05	6.894	8
NM_003149	Src homology three (SH3) and cysteine rich domain	STAC	-5.021	-2.328	1.192	0.509	-7.660	7.29E-05	4.921	10
AK021481	Homo sapiens cDNA FLJ11419 fis		-5.074	-2.343	0.302	0.121	-14.670	1.93E-07	13.330	10
AK021501	Homo sapiens cDNA FLJ11439 fis	LOC92482	-5.134	-2.360	0.701	0.298	-12.770	6.64E-07	11.466	11
AK056438	Kruppel-like factor 4 (gut)		-5.152	-2.365	0.498	0.197	-12.530	7.81E-07	11.212	10
NM_012254	Very long-chain acyl-CoA synthetase homolog 2	SLC27A5	-5.177	-2.372	0.939	0.441	-7.740	6.67E-05	5.012	10
NM_020660	Connexin-36	CX36	-5.216	-2.383	1.774	0.734	-5.232	1.97E-03	0.613	8
NM_001063	Transferrin	TF	-5.260	-2.395	0.630	0.261	-13.820	3.31E-07	12.540	10
NM_003170	Suppressor of Ty 6 homolog (S. cerevisiae)	SUPT6H	-5.267	-2.397	0.788	0.329	-11.600	1.57E-06	10.157	12
NM_005056	Retinoblastoma binding protein 2	RBBP2	-5.329	-2.414	1.566	0.645	-6.139	4.73E-04	2.419	9

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_005471	Glucosamine-6-phosphate isomerase	GNPI	-5.389	-2.430	1.362	0.567	-6.827	1.96E-04	3.533	11
NM_003182	Tachykinin	TAC1	-5.441	-2.444	1.031	0.418	-9.012	1.71E-05	6.897	11
NM_016548	Golgi phosphoprotein 2	GOLPH2	-5.457	-2.448	0.992	0.401	-9.337	1.22E-05	7.375	10
AK022010	Homo sapiens cDNA FLJ11948 fis		-5.479	-2.454	0.632	0.258	-13.500	1.42E-06	10.529	10
NM_032951	Williams Beuren syndrome chromosome region 14	WBSCR14	-5.521	-2.465	0.747	0.303	-12.550	7.77E-07	11.214	12
AK024939	Homo sapiens cDNA: FLJ21286 fis		-5.552	-2.473	1.708	0.692	-5.872	6.85E-04	1.982	8
NM_015904	Translation initiation factor IF2	IF2	-5.571	-2.478	1.209	0.488	-7.962	5.19E-05	5.325	12
AK024873	Homo sapiens cDNA: FLJ21220 fis	NF1	-5.598	-2.485	0.559	0.224	-15.830	9.78E-08	14.363	10
NM_032164	Hypothetical protein FLJ12298	FLJ12298	-5.606	-2.487	0.893	0.359	-10.900	2.83E-06	9.397	9
NM_016623	Hypothetical protein	BM-009	-5.633	-2.494	1.103	0.535	-5.318	1.47E-03	0.804	11
AL359941	Homo sapiens mRNA		-5.641	-2.496	0.916	0.360	-9.251	1.33E-05	7.258	10
AK025558	3-hydroxyisobutyrate dehydrogenase	HIBADH	-5.696	-2.510	0.777	0.276	-8.274	5.44E-05	5.447	8
AK056437	Homo sapiens cDNA FLJ31875 fis	FLJ31875	-5.700	-2.511	1.693	0.684	-5.872	6.85E-04	1.982	8
AK055100	Homo sapiens cDNA FLJ30538 fis		-5.700	-2.511	1.754	0.677	-5.396	1.59E-03	0.909	8
NM_016592	GNAS complex locus	GNAS	-5.724	-2.517	1.424	0.561	-6.862	1.87E-04	3.593	11
NM_018649	H2A histone family	H2AFY2	-5.796	-2.535	0.702	0.274	-12.860	6.23E-07	11.552	11
AB040968	KIAA1535 protein	PKLR	-5.812	-2.539	0.748	0.295	-12.900	6.07E-07	11.585	12
NM_001718	Bone morphogenetic protein 6	BMP6	-5.918	-2.565	0.613	0.241	-15.160	1.40E-07	13.783	10
NM_001967	Eukaryotic translation initiation factor	EIF4A2	-5.950	-2.573	0.476	3.111	-11.080	2.39E-06	9.616	175
AK055143	Homo sapiens cDNA FLJ30581 fis		-6.169	-2.625	1.013	0.387	-10.040	6.13E-06	8.276	11
AK057142	Homo sapiens cDNA FLJ32580 fis		-6.251	-2.644	0.719	0.271	-13.310	4.76E-07	12.056	8
NM_016539	Sirtuin (silent mating type information regulation 2 homolog) 6 (S.cerevisiae)	SIRT6	-6.329	-2.662	1.276	0.479	-8.113	4.46E-05	5.556	12
AK055070	Homo sapiens cDNA FLJ20167 fis		-6.662	-2.736	1.802	0.648	-5.946	6.16E-04	2.115	8
AK057447	Homo sapiens cDNA FLJ32885 fis		-6.864	-2.779	1.459	0.516	-7.311	1.09E-04	4.365	10
AK021970	Homo sapiens cDNA FLJ11908 fis		-6.888	-2.784	1.654	0.598	-6.509	2.88E-04	3.065	9
NM_018111	Hypothetical protein FLJ10490	FLJ10490	-6.931	-2.793	1.862	0.666	-5.975	5.93E-04	2.086	10

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
NM_013317	Lung type-I cell membrane-associated glycoprotein, transcript variant 1	T1A-2	-6.960	-2.799	0.789	0.278	-11.730	1.42E-06	10.331	11
NM_006495	Ecotropic viral integration site 2B	EVI2B	-6.984	-2.804	0.444	0.156	-18.620	1.98E-08	16.510	8
NM_024998	Hypothetical protein FLJ12704	FLJ12704	-7.126	-2.833	0.595	0.205	-15.640	1.06E-07	14.201	8
AK026729	Homo sapiens cDNA: FLJ23076 fis		-7.135	-2.835	1.138	0.395	-9.380	1.19E-05	7.434	10
U06711	Mucin 5, subtypes A and C, tracheobronchial/gastric	MUC5AC	-7.230	-2.854	1.606	0.562	-6.774	2.75E-04	3.161	10
NM_032325	Hypothetical protein MGC11102	MGC11102	-7.280	-2.864	1.338	0.464	-8.247	3.85E-05	5.852	9
NM_018656	Bladder cancer overexpressed protein	SLC35E3	-7.346	-2.877	1.108	0.380	-9.927	6.86E-06	8.161	10
BC007323	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2, 14.5kDa	NDUFC2	-7.454	-2.898	1.758	0.604	-6.625	2.50E-04	3.263	9
NM_003620	Protein phosphatase 1D magnesium-dependent, delta isoform	PPM1D	-7.485	-2.904	1.737	0.601	-6.609	2.54E-04	3.236	9
AK025812	Homo sapiens cDNA: FLJ22159 fis	RAI17	-7.501	-2.907	0.966	0.330	-10.910	2.78E-06	9.395	10
NM_001949	E2F transcription factor 3	E2F3	-7.558	-2.918	1.130	0.390	-9.881	7.25E-06	8.102	10
AK021802	Homo sapiens cDNA FLJ11740 fis	SEMA3D	-7.717	-2.948	1.312	0.440	-8.346	5.07E-05	5.548	9
AF334588	Homo sapiens P25 mRNA		-7.765	-2.957	1.488	0.500	-7.752	6.62E-05	5.031	11
NM_017831	Ring finger protein 125	RNF125	-7.923	-2.986	1.778	0.583	-6.613	2.53E-04	3.242	9
NM_005940	Matrix metalloproteinase 11 (stromelysin 3)	MMP11	-8.095	-3.017	1.642	0.531	-6.777	2.07E-04	3.484	10
NM_052842	BCL2-like 12 (proline rich)	BCL2L12	-8.112	-3.020	1.331	0.441	-8.902	1.89E-05	6.741	11
BC014111	Homo sapiens		-8.123	-3.022	0.654	0.214	-15.740	1.95E-07	13.325	9
BC000549	Homo sapiens clone 24758 mRNA sequence		-8.271	-3.048	1.828	0.608	-6.522	2.83E-04	3.087	9
AL136781	Hypothetical protein DKFZp434N1817	DKFZP434N1817	-8.415	-3.073	1.214	0.398	-9.546	1.02E-05	7.687	9
AL049280	Homo sapiens mRNA	CYorf15B	-8.480	-3.084	0.486	0.158	-21.540	5.35E-09	18.442	9

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
S72422	Dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)	DLST	-8.521	-3.091	1.502	0.486	-8.094	4.55E-05	5.557	11
NM_025057	Hypothetical protein FLJ23189	C14orf45	-8.586	-3.102	1.027	0.333	-10.770	5.38E-06	8.628	8
AK000006	Hypothetical protein		-8.664	-3.115	1.131	0.363	-10.160	5.49E-06	8.458	10
NM_014083	PRO0767 protein	PRO0767	-8.803	-3.138	0.936	0.297	-12.910	6.07E-07	11.628	10
NM_000518	Hemoglobin, beta	HBB	-8.840	-3.144	1.049	0.334	-11.590	1.57E-06	10.150	12
AK026280	Homo sapiens cDNA: FLJ22627 fis		-8.938	-3.160	1.397	0.449	-8.859	1.97E-05	6.774	8
NM_024515	Hypothetical protein MGC4645	MGC4645	-9.145	-3.193	0.862	0.292	-9.234	1.35E-05	7.234	9
NM_020359	Phospholipid scramblase 2	PLSCR2	-9.209	-3.203	1.154	0.356	-10.660	3.48E-06	9.108	9
NM_014392	Neuron-specific protein	D4S234E	-9.292	-3.216	1.012	0.312	-12.040	1.15E-06	10.714	9
AK021678	Homo sapiens cDNA FLJ11616 fis		-9.337	-3.223	1.729	0.521	-7.334	1.06E-04	4.477	8
NM_032810	Hypothetical protein FLJ14600	FLJ14600	-9.383	-3.230	1.231	0.343	-7.588	7.85E-05	4.807	9
AK022239	Homo sapiens cDNA FLJ12177 fis		-9.567	-3.258	1.168	0.355	-10.450	4.14E-06	8.801	11
NM_019066	MAGE-like 2	MAGEL2	-10.063	-3.331	1.126	0.372	-8.589	2.62E-05	6.262	11
AK057237	Homo sapiens cDNA FLJ32675 fis		-10.711	-3.421	1.023	0.270	-9.417	1.14E-05	7.484	9
NM_014968	Pitrilysin metalloproteinase 1	PITRM1	-10.785	-3.431	0.930	0.271	-14.260	2.43E-07	12.950	11
AL109714	Homo sapiens mRNA full length insert cDNA clone	LOC283687	-11.066	-3.468	1.709	0.492	-7.902	5.57E-05	5.368	8
AJ001403	Mucin 5, subtypes A and C, tracheobronchial/gastric	MUC5AC	-11.173	-3.482	1.320	0.357	-7.635	7.51E-05	4.953	8
AL050078	Homo sapiens cDNA FLJ10784 fis		-11.448	-3.517	1.503	0.426	-9.294	1.28E-05	7.346	9
NM_020120	UDP-glucose ceramide glucosyltransferase	UGCGL1	-11.519	-3.526	1.363	0.387	-10.080	5.92E-06	8.303	12
AB037761	KIAA1340 protein	KIAA1340	-11.819	-3.563	2.130	0.596	-6.822	1.97E-04	3.637	8
AB037781	Hypothetical protein FLJ10074	FLJ10074	-12.009	-3.586	1.475	0.409	-9.069	2.46E-05	6.558	8
AK055084	Homo sapiens cDNA FLJ30522 fis		-12.329	-3.624	1.824	0.500	-7.976	5.15E-05	5.443	9
AL109691	Homo sapiens mRNA full length insert cDNA clone		-13.343	-3.738	1.700	0.448	-8.322	5.17E-05	5.550	8

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates used
AK054962	Homo sapiens cDNA FLJ30400 fis		-13.352	-3.739	1.652	0.435	-9.066	1.61E-05	7.064	8
AF086735	Microsomal NAD+-dependent retinol dehydrogenase 4	RODH-4	-13.852	-3.792	1.479	0.387	-9.834	7.62E-06	8.012	11
D21262	Nucleolar and coiled-body phosphprotein	NOLC1	-13.938	-3.801	1.206	0.314	-12.080	1.11E-06	10.741	10
BC011969	Homo sapiens		-14.836	-3.891	1.040	0.266	-14.000	2.87E-07	12.726	9
NM_013355	Protein kinase PKNbeta	PKN3	-14.949	-3.902	1.224	0.311	-11.800	1.37E-06	10.408	11
AK055809	Homo sapiens cDNA FLJ31247 fis		-14.970	-3.904	1.527	0.450	-10.220	8.75E-06	8.022	16
AK023182	CGI-145 protein		-15.012	-3.908	0.979	0.250	-15.400	1.26E-07	13.980	12
AK022334	Homo sapiens cDNA FLJ12272 fis		-17.606	-4.138	1.507	0.364	-10.790	3.12E-06	9.222	11
AK002078	Homo sapiens cDNA FLJ11216 fis		-18.507	-4.210	1.628	0.396	-10.760	3.18E-06	9.181	17
NM_000844	Glutamate receptor	GRM7	-20.294	-4.343	1.559	0.364	-10.730	3.28E-06	9.219	8
AK057806	Homo sapiens cDNA FLJ25077 fis		-22.254	-4.476	1.153	0.256	-15.350	1.29E-07	13.955	8
AF003738	Homo sapiens chromosome 21q22.1 anonymous mRNA sequence		-22.596	-4.498	1.035	0.230	-16.940	5.22E-08	15.274	10
AK024863	Homo sapiens cDNA: FLJ21210 fis	DNCLI2	-24.864	-4.636	0.511	0.106	-23.760	1.63E-09	19.786	10
AB028953	KIAA1030 protein	KIAA1030	-31.363	-4.971	1.101	0.219	-16.000	9.24E-08	14.507	9
NM_015369	TP53TG3 protein	TP53TG3	-33.382	-5.061	1.418	0.277	-12.660	7.25E-07	11.398	8
AJ301580	DMRT-like family A2	DMRTA2	-47.308	-5.564	1.370	0.247	-15.850	9.78E-08	14.381	10
AK024871	Homo sapiens cDNA: FLJ21218 fis	APBB2	-55.599	-5.797	0.833	0.144	-26.150	5.91E-10	20.907	9
BC006317	Homo sapiens solute carrier family 18 (vesicular monoamine), member 1	SLC18A1	-61.393	-5.940	0.739	0.124	-29.460	5.91E-10	20.961	11
BC009393	Homo sapiens HIV-1 Rev binding protein-like	HRBL	-113.300	-6.824	0.935	0.137	-28.070	4.35E-10	21.857	10

Differential Gene Expression Studies of Normal Pelvic Peritoneum in Women with and without Endometriosis

202 genes were found to be significantly up- or down-regulated in the normal pelvic peritoneum of women with endometriosis versus those without (Table IV). 115 genes were up-regulated greater than 2-fold with a p-value <0.01, and 87 were down-regulated greater than two-fold. The most up-regulated gene was A20-binding inhibitor of NF-kappa B activation 2 (NM_024309) with a fold change of +24.268 (log2 ratio 4.601, SD 0.799). Eight replicates were utilized in the analysis, with a p-value of 8.98 x 10^{-7}. The gene most down-regulated, with a fold-change of -43.441 (log2 ratio -5.441, SD 0.578) was Troponin T type 3 (skeletal, fast) (NM_006757). Seven replicates were utilized in its analysis, rendering a p-value of 4.20 x 10^{-8}.

Table IV. Log Ratio Changes of Gene Expression in Normal Pelvic Peritoneum of Women with Endometriosis versus Without.

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
NM_024309	A20-binding inhibitor of NF-kappaB activation 2	TNIP2	24.268	4.601	0.799	0.174	19.290	8.98E-07	12.667	8
AJ420589	Homo sapiens mRNA full length insert cDNA clone		19.495	4.285	1.050	0.245	13.850	1.23E-05	9.234	8
AK025643	Homo sapiens cDNA: FLJ21990 fis		18.817	4.234	0.926	0.217	15.010	6.54E-06	10.090	7
NM_017765	PQ loop repeat containing 2	PQLC2	14.400	3.848	1.498	0.381	8.661	2.95E-04	4.522	6
NM_003215	Tec protein tyrosine kinase	TEC	14.182	3.826	1.047	0.269	10.800	7.24E-05	6.698	7
AL049650	Transglutaminase 3-like	TGM3L	13.288	3.732	1.150	0.304	11.020	6.57E-05	6.937	6
BC014396	Copine IV	CPNE4	12.799	3.678	1.017	0.272	11.080	6.36E-05	6.994	6
NM_001249	Ectonucleoside triphosphate diphosphohydrolase 5	ENTPD5	12.799	3.678	0.802	0.217	15.120	6.14E-06	10.169	7
AK055061	Homo sapiens cDNA FLJ30499 fis		12.441	3.637	0.684	0.209	10.220	1.02E-04	6.091	7
NM_003083	Small nuclear RNA activating complex, polypeptide 2	SNAPC2	11.802	3.561	0.895	0.251	13.420	1.49E-05	8.905	8
NM_015887	Putative peroxisome microbody protein 175.1		10.718	3.422	1.436	0.418	8.359	3.64E-04	4.173	6
AK021930	Homo sapiens cDNA FLJ11868 fis		10.440	3.384	1.343	0.394	8.632	2.97E-04	4.439	7
NM_032293	GTPase activating Rap/RanGAP domain-like 3	GARNL3	10.239	3.356	1.225	0.365	9.354	1.83E-04	5.195	8
AK055705	Homo sapiens cDNA FLJ31143 fis		10.077	3.333	1.054	0.310	10.430	9.01E-05	6.377	6
AK026942	Homo sapiens cDNA: FLJ23289 fis		10.070	3.332	0.677	0.204	16.020	3.70E-06	10.765	7
AL050175	Homo sapiens mRNA		10.028	3.326	1.380	0.415	8.267	3.88E-04	3.967	8
NM_000583	Group-specific component (vitamin D binding protein)	GC	10.000	3.322	0.509	0.152	19.720	8.14E-07	12.883	7
NM_022496	ARP6 actin-related protein 6 homolog (yeast)	ACTR6	9.673	3.274	0.996	0.303	11.280	5.62E-05	7.137	7
AK023446	Aminoadipate-semialdehyde synthase	AASS	9.000	3.170	1.278	0.389	7.626	6.49E-04	3.286	6
NM_032447	Fibrillin 3	FBN3	8.706	3.122	1.261	0.405	8.586	3.04E-04	4.385	7
NM_005701	RNA, U transporter I	RNUT1	8.282	3.050	1.220	0.396	8.744	2.78E-04	4.616	6

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
AB033104	Serine/threonine kinase 36	STK36	8.271	3.048	0.910	0.297	11.620	4.40E-05	7.479	6
NM_005738	ADP-ribosylation factor-like 4A	ARL4	7.857	2.974	1.273	0.428	8.005	4.83E-04	3.652	8
AK021831	Homo sapiens cDNA FLJ11769 fis		7.749	2.954	1.027	0.346	9.798	1.37E-04	5.705	7
AB067501	KIAA1914	KIAA1914	7.413	2.890	1.121	0.384	8.653	2.95E-04	4.463	7
AB051514	KIAA1727 protein		7.301	2.868	0.954	0.326	9.833	1.33E-04	5.786	6
NM_004388	Chitobiase	CTBS	7.180	2.844	0.893	0.314	10.750	7.39E-05	6.612	8
AL080077	Homo sapiens genomic DNA		7.047	2.817	1.464	0.519	6.779	1.33E-03	2.179	6
NM_016932	Sine oculis homeobox homolog 2 (Drosophila)	SIX2	6.964	2.800	1.151	0.405	8.386	3.59E-04	4.204	6
NM_024636	STEAP family member 4	STEAP4	6.945	2.796	1.748	0.743	6.219	3.07E-03	1.116	6
AB037715	KIAA1294 protein		6.816	2.769	0.971	0.348	9.661	1.50E-04	5.563	7
NM_014810	Centrosome-associated protein 350kDa	CAP350	6.797	2.765	1.225	0.436	7.435	7.66E-04	2.989	7
AB014542	Trinucleotide repeat containing 15	TNRC15	6.530	2.707	0.701	0.258	12.280	6.15E-05	7.179	6
NM_006174	Neuropeptide Y receptor Y5	NPY5R	6.516	2.704	1.043	0.376	8.003	4.83E-04	3.749	6
S66666	P53		6.467	2.693	0.821	0.305	11.020	6.57E-05	6.865	8
AL359599	Homo sapiens genomic DNA		6.369	2.671	1.284	0.479	7.209	9.18E-04	2.696	7
AK025527	Homo sapiens cDNA: FLJ21874 fis	INSR	6.251	2.644	1.002	0.376	8.940	2.49E-04	4.786	7
BC012458	Sec1 family domain containing 2	SCFD2	5.959	2.575	0.897	0.347	9.778	1.39E-04	5.684	7
NM_001677	ATPase, Na+/K+ transporting, beta 1 polypeptide	ATP1B1	5.905	2.562	1.401	0.533	6.270	2.17E-03	1.469	6
BC008613	Homo sapiens		5.760	2.526	1.213	0.481	7.108	9.89E-04	2.514	8
AK021484	Homo sapiens cDNA FLJ11422 fis		5.438	2.443	1.310	0.666	6.267	2.17E-03	1.464	13
AK056642	Hypothetical LOC552889		5.400	2.433	1.055	0.428	7.660	6.32E-04	3.274	7
NM_006739	MCM5 minichromosome maintenance deficient 5, cell division cycle 46	MCM5	5.141	2.362	0.996	0.417	8.106	4.43E-04	3.873	6
NM_004964	Histone deacetylase 1	HDAC1	5.102	2.351	1.163	0.498	6.968	1.13E-03	2.378	7
NM_005442	Eomesodermin homolog (Xenopus laevis)	EOMES	5.095	2.349	0.376	0.189	8.361	3.64E-04	4.124	6
NM_005201	Chemokine (C-C motif) receptor 8	CCR8	5.088	2.347	0.814	0.338	8.947	2.48E-04	4.843	6

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
AF016903	Agrin	AGRN	5.021	2.328	0.980	0.410	7.749	5.96E-04	3.438	6
NM_014725	START domain containing 8	STARD8	4.969	2.313	1.111	0.479	7.321	8.35E-04	2.897	6
AK054969	Ubiquitin protein ligase E3 component n-recognin 1	UBR1	4.931	2.302	0.716	0.319	10.340	9.44E-05	6.253	13
NM_003379	Villin 2 (ezrin)	VIL2	4.820	2.269	1.136	0.497	6.892	1.21E-03	2.332	6
AK023730	Chromosome 6 open reading frame 188	C6orf188	4.790	2.260	0.933	0.406	8.181	4.15E-04	3.963	6
AK055644	Zinc finger protein 138	ZNF138	4.754	2.249	0.736	0.323	9.988	1.20E-04	5.943	6
AK056177	Chromosome 20 open reading frame 102	C20orf102	4.717	2.238	1.043	0.463	7.366	8.09E-04	2.900	7
NM_024724	Hypothetical protein FLJ22332	FLJ22332	4.588	2.198	0.852	0.388	8.667	2.95E-04	4.433	8
Y11918	H.sapiens IMAGE cDNA clone 26881		4.547	2.185	0.678	0.310	10.860	7.08E-05	6.789	6
AJ420568	Coiled-coil domain containing 28A	CCDC28A	4.547	2.185	1.087	0.494	6.909	1.19E-03	2.298	7
BC018078	Homo sapiens cDNA clone IMAGE:4793871		4.519	2.176	0.462	0.210	14.000	1.13E-05	9.372	7
AK057215	Homo sapiens cDNA FLJ32653 fis		4.451	2.154	1.085	0.504	6.863	1.24E-03	2.236	7
U72518	Human destrin-2 pseudogene mRNA		4.326	2.113	1.008	0.469	7.221	9.09E-04	2.768	6
BC013114	aarF domain containing kinase 4	ADCK4	4.320	2.111	1.082	0.509	6.804	1.30E-03	2.212	6
NM_024594	Pantothenate kinase 3	PANK3	4.260	2.091	0.648	0.310	10.630	8.05E-05	6.489	8
AB051552	Doublecortin and CaM kinase-like 3	DCAMKL3	4.187	2.066	0.970	0.465	7.199	9.22E-04	2.684	7
AK021840	Homo sapiens cDNA FLJ11778 fis		4.138	2.049	1.047	0.507	6.677	1.46E-03	1.983	7
NM_014465	Sulfotransferase family, cytosolic, 1B, member 1	SULT1B1	4.112	2.040	0.851	0.419	8.020	4.77E-04	3.770	6
NM_006182	Discoidin domain receptor family, member 2	DDR2	4.064	2.023	0.950	0.463	7.091	1.01E-03	2.541	7
AL117574	Homo sapiens mRNA	C20orf80	3.997	1.999	0.848	0.413	7.687	6.22E-04	3.361	6
NM_004334	Bone marrow stromal cell antigen 1	BST1	3.972	1.990	0.503	0.261	14.160	1.02E-05	9.512	13
NM_022829	Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3	SLC13A3	3.939	1.978	0.514	0.256	12.040	3.39E-05	7.847	6
AB011153	Phospholipase C, beta 1 (phosphoinositide-specific)	PLCB1	3.907	1.966	1.072	0.545	6.252	2.20E-03	1.332	8

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
AK024867	Homo sapiens cDNA: FLJ21214 fis		3.795	1.924	0.959	0.495	6.847	1.25E-03	2.271	6
NM_004483	Glycine cleavage system protein H (aminomethyl carrier)	GCSH	3.792	1.923	0.658	0.342	9.650	1.50E-04	5.509	8
NM_058188	Homo sapiens chromosome 21 open reading frame 67	C21orf67	3.763	1.912	0.671	0.340	7.485	7.33E-04	3.053	7
NM_007281	Scrapie responsive protein 1	SCRG1	3.735	1.901	0.900	0.473	7.227	9.05E-04	2.720	7
NM_021624	Histamine receptor H4	HRH4	3.678	1.879	0.982	0.514	6.577	1.60E-03	1.901	6
NM_007112	Thrombospondin 3	THBS3	3.487	1.802	0.536	0.289	10.560	8.35E-05	6.507	12
AB002331	Death associated transcription factor 1	DATF1	3.461	1.791	0.834	0.461	7.367	8.09E-04	2.957	6
NM_016299	Heat shock 70kDa protein 14	HSPA14	3.320	1.731	0.866	0.498	6.815	1.29E-03	2.171	7
AF334710	Homo sapiens pyruvate dehydrogenase kinase, isozyme 4	PDK4	3.274	1.711	0.570	0.329	9.318	1.86E-04	5.199	7
AK055220	Homo sapiens cDNA FLJ30658 fis		3.269	1.709	0.568	0.335	8.930	2.49E-04	4.824	6
NM_018440	Phosphoprotein associated with glycosphingolipid microdomains 1	PAG1	3.184	1.671	0.489	0.284	9.693	1.48E-04	5.642	6
AK023371	Homo sapiens cDNA FLJ13309 fis		3.173	1.666	0.585	0.345	8.523	3.23E-04	4.363	6
NM_006327	Translocase of inner mitochondrial membrane 23 homolog (yeast)	TIMM23	3.080	1.623	0.755	0.463	7.218	9.09E-04	2.709	7
AK025346	Homo sapiens cDNA: FLJ21693 fis		3.074	1.620	0.412	0.226	7.847	5.46E-04	3.458	7
NM_033439	DVS27-related protein	DVS27	3.061	1.614	0.769	0.476	7.010	1.09E-03	2.383	8
BC010942	Acetyl-Coenzyme A acetyltransferase 1 (acetoacetyl Coenzyme A thiolase)	ACAT1	3.004	1.587	0.863	0.535	6.226	2.23E-03	1.406	6
AB033114	AT2 receptor-interacting protein 1	MTSG1	2.959	1.565	0.893	0.571	5.985	2.85E-03	0.996	7
NM_015474	SAM domain and HD domain 1	SAMHD1	2.894	1.533	0.767	0.500	6.671	1.47E-03	1.922	8
AK055770	Spermatogenesis associated 13	SPATA13	2.878	1.525	0.753	0.487	6.657	1.49E-03	1.955	7
NM_003226	Trefoil factor 3 (intestinal)	TFF3	2.864	1.518	0.518	0.341	9.436	1.73E-04	5.326	7
BC010099	Homo sapiens chromosome 16 open reading frame 57	C16orf57	2.842	1.507	0.301	0.203	12.020	3.39E-05	7.792	7
NM_003657	Breast carcinoma amplified sequence 1	BCAS1	2.795	1.483	0.377	0.249	10.760	7.38E-05	6.699	6

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
NM_001881	cAMP responsive element modulator	CREM	2.786	1.478	0.744	0.500	6.646	1.51E-03	1.939	7
NM_015665	Achalasia, adrenocortical insufficiency, alacrimia (Allgrove, triple-A)	AAAS	2.782	1.476	0.663	0.444	7.232	9.04E-04	2.726	7
NM_032638	GATA binding protein 2	GATA2	2.778	1.474	0.772	0.524	6.374	1.96E-03	1.507	8
NM_025080	Asparaginase like 1	ASRGL1	2.747	1.458	0.740	0.510	6.452	1.80E-03	1.727	6
NM_005033	Polymyositis/scleroderma autoantigen 1 (75kD)	PMSCL1	2.728	1.448	0.742	0.512	6.508	1.72E-03	1.696	8
AF339829	Homo sapiens clone IMAGE:609847		2.661	1.412	0.756	0.536	6.229	2.23E-03	1.299	8
NM_032764	Homo sapiens chromosome 9 open reading frame 70	C9orf70	2.617	1.388	0.630	0.454	7.232	9.04E-04	2.677	8
NM_003375	Voltage-dependent anion channel 2	VDAC2	2.569	1.361	0.721	0.528	6.345	2.02E-03	1.518	7
NM_006435	Interferon induced transmembrane protein 2 (1-8D)	IFITM2	2.546	1.348	0.642	0.482	6.836	1.26E-03	2.200	7
AL117477	PHD finger protein 19	PHF19	2.535	1.342	0.643	0.479	6.871	1.23E-03	2.195	8
NM_004893	H2A histone family, member Y	H2AFY	2.466	1.302	0.506	0.389	8.191	4.12E-04	3.876	8
NM_014574	Cell cycle S/G2 nuclear autoantigen	STRN3	2.452	1.294	0.704	0.542	6.150	2.41E-03	1.237	7
M55536	Human glucose transporter pseudogene	GLUT6	2.450	1.293	0.500	0.387	8.230	4.00E-04	3.923	8
NM_052960	Retinoid binding protein 7	CRBPIV	2.400	1.263	0.479	0.380	8.320	3.72E-04	4.029	8
AB020691	KIAA0884 protein, GTPase activating Rap/RanGAP domain-like 1	GARNL1	2.367	1.243	0.536	0.419	7.063	1.04E-03	2.559	6
L20860	Glycoprotein Ib (platelet), beta polypeptide	GP1BB	2.362	1.240	0.494	0.389	7.609	6.56E-04	3.264	6
NM_001681	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	ATP2A2	2.291	1.196	0.590	0.492	6.638	1.51E-03	1.928	7
NM_001937	Dermatopontin	DPT	2.274	1.185	0.353	0.301	9.603	1.55E-04	5.503	7
BC012899	Homo sapiens sialidase 4, mRNA		2.250	1.170	0.431	0.366	8.321	3.72E-04	4.129	6
AB011539	EGF-like-domain, multiple 3	EGFL3	2.202	1.139	0.327	0.284	9.569	1.60E-04	5.466	7
NM_001773	CD34 antigen	CD34	2.141	1.098	0.446	0.406	7.672	6.26E-04	3.240	8
AL390172	Branched chain aminotransferase 1, cytosolic	BCAT1	2.116	1.081	0.294	0.316	6.828	1.27E-03	2.138	7

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
NM_021023	Complement factor H-related 3	FHR-3	2.076	1.054	0.459	0.439	7.163	9.45E-04	2.692	6
NM_016651	Heptacellular carcinoma novel gene 3	DACT1	2.025	1.018	0.486	0.477	6.640	1.51E-03	1.880	8
AF064238	Smoothelin	SMTN	-2.010	-1.007	0.279	0.277	-9.903	1.28E-04	5.771	8
AK056438	Homo sapiens cDNA FLJ31876 fis		-2.020	-1.014	0.469	0.462	-6.459	1.79E-03	1.736	6
NM_030964	Sprouty homolog 4 (Drosophila)	SPRY4	-2.029	-1.021	0.324	0.317	-9.085	2.22E-04	4.902	8
NM_005418	Suppression of tumorigenicity 5	ST5	-2.031	-1.022	0.264	0.258	-10.400	9.09E-05	6.267	8
NM_005271	Glutamate dehydrogenase 1	GLUD1	-2.035	-1.025	0.439	0.431	-7.234	9.04E-04	2.728	7
AK021643	Homo sapiens cDNA FLJ11581 fis		-2.066	-1.047	0.221	0.205	-8.466	3.41E-04	4.246	7
AL049471	Homo sapiens mRNA		-2.086	-1.061	0.469	0.447	-6.984	1.11E-03	2.399	7
AK023496	Homo sapiens cDNA FLJ13434 fis		-2.092	-1.065	0.497	0.470	-6.215	2.26E-03	1.390	6
NM_001614	Actin, gamma 1	ACTG1	-2.110	-1.077	0.392	0.364	-8.346	3.66E-04	4.060	8
AL157773	Human DNA sequence from clone RP11-345L2 on chromosome 6		-2.117	-1.082	0.411	0.378	-7.683	6.22E-04	3.356	6
AK056574	Homo sapiens cDNA FLJ32012 fis		-2.144	-1.100	0.399	0.362	-8.413	3.55E-04	4.139	8
NM_003877	STAT induced STAT inhibitor-2	SOCS2	-2.155	-1.108	0.495	0.433	-6.708	1.42E-03	2.081	6
BG178211	Homo sapiens mesenchymal stem cell protein		-2.155	-1.108	0.582	0.511	-5.969	2.89E-03	1.032	6
AL137547	Homo sapiens mRNA		-2.155	-1.108	0.527	0.473	-6.776	1.33E-03	2.174	6
AK057363	Homo sapiens cDNA FLJ32801 fis		-2.166	-1.115	0.427	0.389	-7.912	5.21E-04	3.639	6
AB020626	KIAA0819 protein	KIAA0819	-2.173	-1.120	0.435	0.385	-7.876	5.40E-04	3.542	7
AK023300	Homo sapiens cDNA FLJ13238 fis		-2.215	-1.147	0.404	0.352	-8.691	2.90E-04	4.460	8
AL137798	Human DNA sequence from clone RP5-1182A14 on chromosome 1		-2.225	-1.154	0.465	0.403	-7.806	5.65E-04	3.408	8
NM_001375	Deoxyribonuclease II, lysosomal	DNASE2	-2.236	-1.161	0.385	0.334	-8.917	2.50E-04	4.809	6
NM_022154	Up-regulated by BCG-CWS	BIGM103	-2.241	-1.164	0.412	0.354	-8.659	2.95E-04	4.423	8
NM_025004	Homo sapiens coiled-coil domain containing 15	CCDC15	-2.245	-1.167	0.452	0.382	-7.548	6.95E-04	3.187	6
NM_016224	Sorting nexin 9	SNX9	-2.256	-1.174	0.525	0.450	-7.168	9.43E-04	2.643	7
NM_001102	Actinin, alpha 1	ACTN1	-2.261	-1.177	0.229	0.176	-8.819	2.62E-04	4.605	7

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
AK022930	Homo sapiens cDNA FLJ12868 fis	YME1L1	-2.267	-1.181	0.546	0.462	-6.992	1.11E-03	2.359	8
AK023302	Homo sapiens cDNA FLJ13240 fis		-2.270	-1.183	0.395	0.324	-8.212	4.04E-04	4.000	6
AL137340	Hypothetical protein DKFZp761C1711		-2.277	-1.187	0.452	0.376	-8.155	4.24E-04	3.932	6
AB029031	TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1	TBC1D1	-2.294	-1.198	0.582	0.487	-6.731	1.40E-03	2.057	7
NM_003289	Tropomyosin 2 (beta)	TPM2	-2.313	-1.210	0.452	0.374	-8.362	3.64E-04	4.078	8
NM_017668	LIS1-interacting protein NUDE1	NDE1	-2.344	-1.229	0.522	0.425	-7.546	6.95E-04	3.082	8
NM_000191	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase	HMGCL	-2.397	-1.261	0.650	0.529	-6.258	2.18E-03	1.452	6
NM_024999	Hypothetical protein FLJ12988	FLJ12988	-2.398	-1.262	0.492	0.390	-8.162	4.22E-04	3.842	8
AB026156	Homo sapiens TCAM-1 pseudogene mRNA for testicular cell adhesion molecule 1		-2.452	-1.294	0.693	0.535	-6.251	2.20E-03	1.383	7
NM_024018	Butyrophilin, subfamily 2, member A3	BTN2A3	-2.466	-1.302	0.517	0.396	-8.119	4.38E-04	3.889	6
NM_003135	Signal recognition particle 19kDa	SRP19	-2.486	-1.314	0.469	0.356	-8.853	2.58E-04	4.689	7
AK021501	Homo sapiens cDNA FLJ11439 fis	LOC92482	-2.512	-1.329	0.486	0.365	-8.668	2.95E-04	4.434	8
NM_001718	Bone morphogenetic protein 6	BMP6	-2.528	-1.338	0.509	0.384	-8.279	3.84E-04	4.079	6
AK021481	Homo sapiens cDNA FLJ11419 fis		-2.528	-1.338	0.371	0.277	-10.810	7.24E-05	6.666	8
AF109681	Integrin, alpha 11	ITGA11	-2.569	-1.361	0.630	0.459	-6.958	1.14E-03	2.421	6
NM_030570	Uroplakin 3B	UPK3B	-2.594	-1.375	0.725	0.527	-6.310	2.09E-03	1.415	8
AB027233	19A24 protein	SLAMF7	-2.614	-1.386	0.336	0.242	-12.020	3.39E-05	7.759	8
NM_015906	Tripartite motif-containing 33	TRIM33	-2.621	-1.390	0.257	0.185	-14.330	9.46E-06	9.585	8
AF176707	F-box only protein 29	FBXO29	-2.757	-1.463	0.506	0.346	-9.236	1.99E-04	5.067	8
NM_022873	Interferon, alpha-inducible protein (clone IFI-6-16)	G1P3	-2.789	-1.480	0.646	0.436	-7.547	6.95E-04	3.082	8
NM_005877	Splicing factor 3a, subunit 1, 120kDa	SF3A1	-2.797	-1.484	0.504	0.334	-8.995	2.36E-04	4.896	6
AK055600	Popeye protein 3, Homo sapiens cDNA FLJ31038 fis		-2.825	-1.498	0.659	0.439	-7.511	7.17E-04	3.037	8
NM_024998	Hypothetical protein FLJ12704	FLJ12704	-2.856	-1.514	0.823	0.546	-6.235	2.23E-03	1.360	7
AB040968	KIAA1535 protein	KIAA1535	-2.870	-1.521	0.281	0.166	-8.029	4.76E-04	3.729	6

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
NM_021238	TERA protein, Family with sequence similarity 60, member A	TERA	-2.965	-1.568	0.427	0.278	-9.695	1.48E-04	5.644	6
NM_016564	BM88 antigen	BM88	-2.969	-1.570	0.642	0.405	-7.870	5.41E-04	3.534	7
BC002770	Homo sapiens, clone IMAGE:3616574	LOC92154	-3.008	-1.589	0.759	0.478	-6.986	1.11E-03	2.351	8
AL080232	Homo sapiens genomic DNA		-3.276	-1.712	0.660	0.387	-8.598	3.02E-04	4.399	7
NM_001610	Acid phosphatase 2, lysosomal	ACP2	-3.437	-1.781	0.879	0.506	-6.700	1.43E-03	2.071	6
NM_003809	Tumor necrosis factor (ligand) superfamily, member 12	TNFSF12	-3.568	-1.835	0.923	0.503	-6.728	1.41E-03	2.001	8
NM_001276	Chitinase 3-like 1 (cartilage glycoprotein-39)	CHI3L1	-3.590	-1.844	0.574	0.315	-9.304	1.88E-04	5.232	6
NM_005855	Receptor (calcitonin) activity modifying protein 1	RAMP1	-3.613	-1.853	0.535	0.289	-11.190	5.94E-05	7.057	7
NM_003186	Transgelin	TAGLN	-3.625	-1.858	0.360	0.216	-9.696	1.48E-04	5.557	7
NM_030765	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 4	B3GNT4	-3.655	-1.870	0.394	0.210	-14.270	9.68E-06	9.570	7
NM_013271	Proprotein convertase subtilisin/kexin type 1 inhibitor	PCSK1N	-3.655	-1.870	0.637	0.341	-9.654	1.50E-04	5.513	8
AL110248	Homo sapiens mRNA		-3.789	-1.922	0.279	0.164	-9.398	1.79E-04	5.242	7
NM_030655	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL-1 like helicase homolog, S. cerevisiae)	DDX11	-4.003	-2.001	1.024	0.512	-6.634	1.52E-03	1.872	8
NM_001299	Calponin 1, basic, smooth muscle	CNN1	-4.441	-2.151	0.924	0.424	-7.428	1.11E-03	2.570	6
NM_032454	Serine/threonine kinase 19	STK19	-4.604	-2.203	0.735	0.333	-10.060	1.14E-04	5.976	7
NM_053025	Myosin, light polypeptide kinase	MYLK	-4.617	-2.207	0.593	0.269	-12.110	3.35E-05	7.834	8
NM_001613	Actin, alpha 2, smooth muscle, aorta	ACTA2	-4.807	-2.265	1.093	0.475	-6.893	1.21E-03	2.276	7
AB051448	KIAA1661 protein	KIAA1661	-4.928	-2.301	0.338	0.170	-8.930	2.49E-04	4.775	6
AK025094	Homo sapiens cDNA: FLJ21441 fis	ACTA2	-5.000	-2.322	0.897	0.392	-8.694	2.90E-04	4.559	6
NM_013317	Lung type-I cell membrane-associated glycoprotein	T1A-2	-5.021	-2.328	0.356	0.174	-9.853	1.31E-04	5.762	6
NM_014392	Neuron-specific protein	D4S234E	-5.032	-2.331	0.792	0.347	-9.503	1.68E-04	5.444	6

Gene ID	Description	Symbol	Fold Change	Average log2 ratio	SD	CV	t-test	P-value	B-statistic	Replicates Used
NM_017680	Asporin (LRR class 1)	ASPN	-5.148	-2.364	1.079	0.460	-7.459	7.51E-04	3.020	7
AL359941	Homo sapiens mRNA		-5.864	-2.552	0.600	0.266	-8.639	2.96E-04	4.401	7
U11058	Potassium large conductance calcium-activated channel, subfamily M, alpha member 1	KCNMA1	-5.905	-2.562	0.705	0.275	-12.080	3.38E-05	7.812	8
NM_022844	Myosin, heavy polypeptide 11, smooth muscle	MYH11	-5.905	-2.562	0.684	0.267	-12.380	2.87E-05	8.064	8
NM_000300	Phospholipase A2, group IIA (platelets, synovial fluid)	PLA2G2A	-5.971	-2.578	0.530	0.206	-15.600	4.79E-06	10.469	8
NM_002923	Regulator of G-protein signalling 2, 24kDa	RGS2	-6.285	-2.652	0.932	0.356	-9.213	2.02E-04	5.086	7
NM_000867	5-hydroxytryptamine (serotonin) receptor 2B	HTR2B	-6.360	-2.669	0.655	0.246	-13.600	1.40E-05	9.097	6
NM_001901	Connective tissue growth factor	CTGF	-6.998	-2.807	0.459	0.163	-19.180	9.28E-07	12.608	8
NM_005909	Microtubule-associated protein 1B	MAP1B	-7.003	-2.808	0.787	0.281	-12.010	3.39E-05	7.786	7
NM_018649	H2A histone family, member Y2	H2AFY2	-7.140	-2.836	0.299	0.114	-15.250	5.84E-06	10.255	6
AK055872	Haptoglobin	HP	-7.428	-2.893	0.835	0.289	-11.650	4.34E-05	7.438	8
NM_001615	Actin, gamma 2, smooth muscle, enteric	ACTG2	-7.574	-2.921	0.986	0.334	-9.964	1.22E-04	5.919	6
AK023182	CGI-145 protein	CGI-145	-10.447	-3.385	1.073	0.317	-10.880	7.02E-05	6.771	7
NM_006198	Purkinje cell protein 4	PCP4	-11.624	-3.539	1.596	0.453	-7.738	6.01E-04	3.371	7
NM_014456	Programmed cell death 4 (neoplastic transformation inhibitor)	PDCD4	-14.980	-3.905	0.885	0.227	-14.880	6.82E-06	9.980	8
NM_024567	Hypothetical protein FLJ21616	FLJ21616	-20.606	-4.365	0.847	0.194	-17.680	1.63E-06	11.773	6
NM_006495	Ecotropic viral integration site 2B	EVI2B	-25.246	-4.658	0.302	0.062	-23.590	2.74E-07	14.695	7
U87460	G protein-coupled receptor 37 (endothelin receptor type B-like protein mRNA)		-38.160	-5.254	0.654	0.125	-26.990	9.34E-08	15.879	6
NM_006757	Troponin T type 3 (skeletal, fast)	TNNT3	-43.441	-5.441	0.578	0.106	-30.430	4.20E-08	17.058	7

RT-PCR Validation Studies

RT-PCR validation of the eutopic endometrium microarrays included utilization of 5 individual gene primer sets and comparing the abundance of the gene of interest compared to GAPDH. Three separate RT-PCR experiments were conducted with each cDNA for each gene. Within the eutopic endometrium samples, the average fold-changes of the genes were as follows: Mammoglobin, 37.908 (Table V); Thrombospondin, 1.267 (Table VI); GADD45B, 3.133 (Table VII); G2 & S phase expressed, 1.488 (Table VIII); and Homo sapiens BC009393, 0.677 (Table IX).

Validation studies for the normal pelvic peritoneum microarray studies included RT-PCR utilizing three separate gene primer sets and comparing their abundance to GAPDH. Three separate experiments were conducted with each cDNA for each gene. The genes had the following fold-changes: SOCS2, 2.240 (Table X); MCM5, 2.877 (Table XI); and TNIP2, 3.833 (Table XII).

Table V. RT-PCR Studies of Mammoglobin.

Sample ID	GADPH Ct	Mammoglobin Ct	Gene/Control*1000	Average	SD	Fold Change	Ave Fold Change
Pooled EA-1	18.33	28.22	1.054	1.051	0.239		**37.908**
Pooled EA-2	18.60	28.20	1.289				
Pooled EA-3	18.81	29.08	0.810				
E03 EA-1	16.76	27.60	0.546	0.594	0.061	0.565	
E03 EA-2	19.40	30.17	0.573				
E03 EA-3	17.24	27.80	0.662				
E04 EA-1	13.48	25.22	0.292	0.262	0.074	0.249	
E04 EA-2	16.26	28.72	0.177				
E04 EA-3	15.57	27.20	0.316				
E05 EA-1	16.53	23.17	10.027	10.407	1.778	9.904	
E05 EA-2	17.19	23.53	12.344				
E05 EA-3	16.90	23.72	8.851				
E11 EA-1	17.26	21.12	68.869	68.624	6.865	65.306	
E11 EA-2	16.71	20.44	75.363				
E11 EA-3	16.65	20.67	61.640				
E15 EA-1	16.66	22.09	23.196	40.955	16.279	38.975	
E15 EA-2	16.67	20.85	55.169				
E15 EA-3	16.40	20.89	44.502				
E17 EA-1	15.70	18.87	111.105	118.160	7.931	112.448	
E17 EA-2	15.71	18.81	116.629				
E17 EA-3	15.87	18.85	126.745				

Table VI. RT-PCR Studies of Thrombospondin 3.

Sample ID	GADPH Ct	Thrombospondin Ct	Gene/Control*1000	Average	SD	Fold Change	Ave Fold Change
Pooled EA-1	18.33	25.29	8.032	7.104	0.820		**1.267**
Pooled EA-2	18.60	25.80	6.801				
Pooled EA-3	18.81	26.08	6.479				
E03 EA-1	16.76	23.70	8.144	11.821	5.971	1.664	
E03 EA-2	19.40	25.14	18.711				
E03 EA-3	17.24	24.10	8.609				
E04 EA-1	13.48	24.64	0.437	3.737	3.232	0.526	
E04 EA-2	16.26	24.27	3.879				
E04 EA-3	15.57	22.75	6.896				
E05 EA-1	16.53	23.45	8.258	8.313	2.040	1.170	
E05 EA-2	17.19	24.50	6.302				
E05 EA-3	16.90	23.49	10.380				
E11 EA-1	17.26	24.05	9.037	7.004	2.498	0.986	
E11 EA-2	16.71	24.60	4.216				
E11 EA-3	16.65	23.66	7.759				
E15 EA-1	16.66	22.70	15.198	15.723	3.151	2.213	
E15 EA-2	16.67	22.38	19.104				
E15 EA -3	16.40	22.68	12.869				
E17 EA-1	15.70	22.67	7.977	7.387	0.532	1.040	
E17 EA-2	15.71	22.82	7.239				
E17 EA-3	15.87	23.04	6.944				

Table VII. RT-PCR Studies of Growth Arrest and DNA-Damage-Inducible, beta.

Sample ID	GADPH Ct	GADD45B Ct	Gene/Control*1000	Average	SD	Fold Change	Ave Fold Change
Pooled EA-1	18.60	26.48	4.245	4.736	0.694		**3.133**
Pooled EA-2	18.81	26.39	5.226				
E03 EA-1	16.76	22.53	18.326	22.856	3.924	4.826	
E03 EA-2	19.40	24.72	25.033				
E03 EA-3	17.24	22.55	25.208				
E04 EA-1	13.48	22.23	2.323	11.641	8.634	2.458	
E04 EA-2	16.26	21.95	19.370				
E04 EA-3	15.57	21.81	13.230				
E05 EA-1	16.53	24.09	5.299	4.993	0.918	1.054	
E05 EA-2	17.19	24.64	5.719				
E05 EA-3	16.90	24.88	3.961				
E11 EA-1	17.26	25.37	3.619	4.638	2.912	0.979	
E11 EA-2	16.71	23.69	7.922				
E11 EA-3	16.65	25.37	2.371				
E15 EA-1	16.66	21.92	26.096	20.348	5.239	4.297	
E15 EA-2	16.67	22.38	19.104				
E15 EA -3	16.40	22.38	15.843				
E17 EA-1	15.70	21.19	22.251	24.544	4.914	5.183	
E17 EA-2	15.71	21.27	21.197				
E17 EA-3	15.87	20.92	30.186				

Table VIII. RT-PCR Studies of G2 and S Phase Expressed 1.

Sample ID	GADPH Ct	G2 & S Phase Expressed Ct	Gene/Control*1000	Average	SD	Fold Change	Ave Fold Change
Pooled EA-1	18.33	27.67	1.543	2.180	0.551		**1.488**
Pooled EA-2	18.60	27.25	2.489				
Pooled EA-3	18.81	27.45	2.507				
E03 EA-1	16.76	23.78	7.705	16.090	12.280	7.382	
E03 EA-2	19.40	24.45	30.186				
E03 EA-3	17.24	23.83	10.380				
E04 EA-1	13.48	25.08	0.322	0.414	0.148	0.190	
E04 EA-2	16.26	27.00	0.585				
E04 EA-3	15.57	27.11	0.336				
E05 EA-1	16.53	26.89	0.761	0.766	0.053	0.351	
E05 EA-2	17.19	27.64	0.715				
E05 EA-3	16.90	27.15	0.821				
E11 EA-1	17.26	27.75	0.695	0.690	0.106	0.316	
E11 EA-2	16.71	27.46	0.581				
E11 EA-3	16.65	26.95	0.793				
E15 EA-1	16.66	27.40	0.585	1.037	0.422	0.476	
E15 EA-2	16.67	26.13	1.420				
E15 EA -3	16.40	26.22	1.106				
E17 EA-1	15.70	27.85	0.220	0.460	0.220	0.211	
E17 EA-2	15.71	26.29	0.653				
E17 EA-3	15.87	26.82	0.506				

Table IX. RT-PCR Studies of Homo sapiens BC009393.

Sample ID	GADPH Ct	Homo BC009393 Ct	Gene/Control*1000	Average	SD	Fold Change	Ave Fold Change
Pooled EA-1	18.33	30.16	0.275	0.745	0.440		**0.677**
Pooled EA-2	18.60	28.86	0.816				
Pooled EA-3	18.81	28.58	1.145				
E03 EA-1	16.76	25.77	1.940	1.459	1.210	1.958	
E03 EA-2	19.40	32.95	0.083				
E03 EA-3	17.24	25.97	2.355				
E04 EA-1	16.26	33.26	0.008	0.010	0.003	0.013	
E04 EA-2	15.57	31.97	0.012				
E05 EA-1	16.53	26.36	1.099	0.816	0.620	1.095	
E05 EA-2	17.19	30.40	0.106				
E05 EA-3	16.90	26.55	1.245				
E11 EA-1	17.26	31.66	0.046	0.415	0.338	0.557	
E11 EA-2	16.71	27.17	0.710				
E11 EA-3	16.65	27.65	0.488				
E15 EA-1	16.66	30.79	0.056	0.294	0.346	0.394	
E15 EA-2	16.67	27.17	0.691				
E15 EA -3	16.40	29.26	0.135				
E17 EA-1	15.70	30.14	0.045	0.032	0.013	0.042	
E17 EA-2	15.71	31.46	0.018				
E17 EA-3	15.87	30.81	0.032				

Table X. RT-PCR Studies of Suppressor of Cytokine Signaling 2.

Sample ID	GADPH Ct	SOCS2 Ct	Gene/Control*1000	Average	SD	Fold Change	Ave Fold Change
Pooled NPP-1	22.68	25.98	101.532	92.287	13.073		**2.240**
Pooled NPP-2	22.30	25.89	83.043				
E03 EA-1	24.26	25.92	316.439	359.654	56.338	3.897	
E03 EA-2	24.94	26.18	423.373				
E03 EA-3	25.44	27.00	339.151				
E04 EA-1	22.82	27.48	39.555	87.524	44.560	0.948	
E04 EA-2	23.29	26.26	127.627				
E04 EA-3	23.31	26.70	95.391				
E09 EA-1	23.32	24.90	334.482	172.884	141.340	1.873	
E09 EA-2	23.29	27.08	72.293				
E09 EA-3	22.72	25.88	111.878				

Table XI. RT-PCR Studies of Minichromosome Maintenance Deficient 5, Cell Division Cycle 46.

Sample ID	GADPH Ct	MCM5 Ct	Gene/Control*1000	Average	SD	Fold Change	Ave Fold Change
Pooled NPP-1	22.68	28.52	17.458	18.686	1.738		**2.877**
Pooled NPP-3	24.29	29.94	19.915				
E03 EA-1	24.26	27.96	76.947	80.709	25.684	4.319	
E03 EA-2	24.94	29.07	57.114				
E03 EA-3	25.44	28.65	108.067				
E04 EA-1	22.82	28.66	17.458	34.921	15.316	1.869	
E04 EA-2	23.29	27.89	41.235				
E04 EA-3	23.31	27.75	46.071				
E09 EA-1	23.32	27.14	70.805	45.628	21.815	2.442	
E09 EA-2	23.29	28.18	33.726				
E09 EA-3	22.72	27.67	32.352				

Table XII. RT-PCR Studies of A20-Binding Inhibitor of NF-kappaB Activation 2.

Sample ID	GADPH Ct	TNIP2 Ct	Gene/Control*1000	Average	SD	Fold Change	Average Fold Change
Pooled NPP-1	22.68	25.85	111.105	197.398	166.725		**3.833**
Pooled NPP-2	22.30	25.75	91.505				
Pooled NPP-3	24.29	25.65	389.582				
E03 EA-1	24.94	24.07	1827.663	1760.577	94.874	8.919	
E03 EA-2	25.44	24.68	1693.491				
E04 EA-1	22.82	25.13	201.660	240.597	36.325	1.219	
E04 EA-2	23.29	25.31	246.558				
E04 EA-3	23.31	25.18	273.573				
E09 EA-1	23.32	25.33	248.273	268.828	160.190	1.362	
E09 EA-2	23.29	24.48	438.303				
E09 EA-3	22.72	25.78	119.908				

CHAPTER V

DISCUSSION

In keeping with Sampson's theory of retrograde menstruation as the source for the cells that make up endometriotic lesions, it would be expected that the eutopic endometrial cells and/or the pelvic peritoneum of women with endometriosis would display properties of increased adhesion. In addition, cell growth and apoptotic pathways would be altered to allow for the continued presence of these cells in the peritoneal cavity rather than their destruction. Cells involved in inflammatory response and cytotoxicity, such as macrophages, would be expected to be deficient in their capability to carry out phagocytosis and cytolysis in the peritoneal environment.

Pathway Involvement

Importing of genes from the eutopic endometrium data set into the Kyoto Encyclopedia of Genes and Genomes (KEGG) rendered involvement of differentially expressed genes in 103 pathways. The pathways in which differentially expressed genes were most involved included: Cell Communication; MAPK Signaling Pathway; Cytokine-Cytokine Receptor Interaction; Cell Cycle; TGF-beta Signaling Pathway; Focal Adhesion; ECM-Receptor Interaction; Cell Adhesion Molecules; Adherens, Tight, and Gap Junctions; and Leukocyte Transendothelial Migration.

Differentially expressed genes in the normal pelvic peritoneum data set were involved in 59 KEGG Pathways. The pathways with the greatest representation of genes included: Calcium Signaling Pathway; Cytokine-Cytokine Receptor Interaction; Cell Cycle; TGF-beta Signaling Pathway; Focal Adhesion; Leukocyte Transendothelial Migration; Regulation of Actin Cytoskeleton; ECM-Receptor Interaction; and Adherens, Tight, and Gap Junctions.

Several genes involved in cell adhesion were found to be up-regulated in the eutopic endometrium of women with endometriosis. These include junctional adhesion molecule I (*F11R*) and thrombospondin 3 (*THBS3* or *TSP3*). *F11R* was up-regulated 3.782-fold ($p=5.12 \times 10^{-7}$) and is involved in the following KEGG pathways: Cell Adhesion Molecules, Tight Junctions, and Leukocyte Transendothelial Migration. *F11R* is found on the surface of human platelets and has been suggested to play a role in the adhesion of platelets to cytokine-inflamed endothelial cells. It is also an important regulator of tight junction assembly in epithelial cells and is involved in platelet aggregation, secretion, adhesion, and spreading (111). This might aid in the adhesion of endometrial cells to the pelvic peritoneum, as well as participate in vasculogenesis with implanting endometriotic lesions.

THBS3, which was up-regulated 3.422-fold ($p=5.48 \times 10^{-4}$) in the eutopic endometrium of women with endometriosis, was also found to be up-regulated in the normal pelvic peritoneum of women with endometriosis (+3.487, $p=8.35 \times 10^{-5}$). In both studies, *THBS3* was involved in the Cell Communication, TGF-beta Signaling, Focal Adhesion, ECM-Receptor Interaction KEGG pathways. *THBS3* is a member of a family of extracellular matrix glycoproteins that mediate interactions between cells and the

extracellular matrix. It is located at chromosome 1q21-24. *THBS3* has been found in high abundance in endocrine tissues (pituitary, testis, thyroid, adrenal, thymus, and stomach), muscle (uterus, colon, prostate, and bladder), and fetal tissues (kidney and lung). In addition, high levels of *THBS3* have been found in placenta and mammary gland tissue, while the expression of *THBS3* mRNA has been characterized as low in the ovary and fetal thymus. Other members of the thrombospondin family, including *TSP1* and *TSP4*, have shown expression in connective tissue and been found to bind to extracellular matrix proteins including heparin sulfate proteoglycan, collagen, fibronectin, and laminin. In addition, *TSP1* binds to *TGF-β1* (112).

Other genes involved in cell adhesion were found to be down-regulated in the eutopic endometrium of women with endometriosis, including β-catenin, *GNAS* complex locus, Connexin-36, and Phosphatase and tensin homolog. Interestingly, down-regulated expression of β-catenin decreases *TGF-β* induced apoptosis (113). This may be one way in which endometrial cells in women with endometriosis are able to survive longer in the intrauterine environment, and then have a greater chance of being refluxed and subsequently involved in attachment to the peritoneal lining and generation of an endometriotic implant.

Tumor necrosis factor (ligand) superfamily, member 12 (*TNFSF12*), was found to be down-regulated 2.604-fold ($p=2.56 \times 10^{-6}$) in the eutopic endometrium of women with endometriosis; it was down-regulated in normal pelvic peritoneum as well (-3.568, $p=1.41 \times 10^{-3}$). *TNFSF12* is implicated in the cytokine-cytokine receptor interaction KEGG pathway, alongside bone morphogenetic protein 2, chemokine ligands, and interleukin receptors. *TNFSF12*, also known as *TWEAK* (*TNF*-related weak inducer of

apoptosis), has been found in various tissues and cells and has been associated with endothelial cell proliferation and angiogenesis. It has been shown to lead to cell death via interactions with caspases leading to apoptosis, as well as with cathepsin B, leading to cell necrosis (114). Its down-regulation in both the eutopic endometrium and normal pelvic peritoneum of women with endometriosis may allow for continued growth of cells that would typically undergo death via a *TWEAK*-induced pathway. Although an increase in *TWEAK* could be proposed to aid in proliferation of endometriotic cells and vasculature, *TWEAK*'s role in endometriosis appears to be more associated with cell viability. In addition, *TWEAK* is located at chromosome 17p13, a region that has been found to have loss of heterozygosity in late-stage endometriosis (91) and to contain the gene for *TP53* (87).

Only one study involving microarray analysis and gene expression in endometriosis has incorporated KEGG pathways into the data analysis. Wu et al. compared expression of eutopic endometrium versus ovarian and nonovarian endometriosis utilizing cDNA microarrays imprinted with 9600 genes. Over 900 genes were differentially expressed and were involved in 79 KEGG pathways. The pathways represented were: Purine Metabolism; Glutathione Metabolism; Starch and Sucrose Metabolism; MAPK Signaling Pathway; Calcium Signaling Pathway; Cytokine-Cytokine Receptor Interaction; Cell Cycle; Apoptosis; Wnt Signaling Pathway; TGF-beta Signaling Pathway; Focal Adhesion; Tight Junction; Toll-like Receptor Signaling Pathway; Jak-STAT Signaling Pathway; and Regulation of Actin Cytoskeleton. In comparing the results of the present study with those of Wu et al., differentially expressed genes in the eutopic endometrium portion of this study were present in all of the

aforementioned pathways. In addition, the proportion of genes involved were similar. However, the microarrays contained only two genes that were identical and were differentially expressed in both studies: *MKNK1* in the MAPK Signaling Pathway and *BMP6* in the TGF-beta Signaling Pathway. The lack of overlap could be for several reasons, most likely due to the utilization of different arrays with a different number and selection of genes. In addition, the Wu study was a comparison of eutopic versus ectopic endometrium in women with endometriosis, whereas this study compared eutopic endometrium in women with and without endometriosis. Lastly, despite the aforementioned reasons for differences, it is promising that both studies show differential expression of genes in many of the same metabolic pathways.

Comparison of Results to Prior Microarray Studies

The most appropriate studies to compare the results of the present study to are those examining differences in gene expression of eutopic endometrium in women with and without endometriosis. In regard to the study by Chen et al., there were similarities in differential gene expression involving transforming growth factor-β, neuropeptide Y receptor, ras-related genes, and keratin-associated proteins. However, the results from the Chen study were not reported in a formal research paper; therefore, it is difficult to compare gene-to-gene. In addition, whether genes were up-regulated or down-regulated was not specified (6).

Differential expression of heat shock proteins and tyrosine-related genes was demonstrated in this study and that of Ezeh et al. Heat shock 70kD protein 1B had a significantly higher expression in the endometrium of women with endometriosis in the

Ezeh study. In the present study, another heat shock 70kDa protein, 9B, was found to be down-regulated 2.205-fold ($p=3.35 \times 10^{-4}$) in endometrium, while in the normal pelvic peritoneum, heat shock 70kDa protein 14 was up-regulated 3.320-fold (p-value=1.29×10^{-3}). Tyrosine 3-monooxygenase was significantly down-regulated in women with endometriosis in the Ezeh study (106). Several tyrosine kinase genes were differentially expressed in the present study; however, none of them were tyrosine monooxygenase genes.

The most comprehensive study of eutopic endometrium gene expression was conducted by Kao et al. (107) and compared expression in women with and without endometriosis during the window of implantation. These results were compared with a prior study by Kao et al. (109) which examined differences in eutopic endometrial gene expression in nonpregnant women between 28 and 39 yr of age with regular menstrual cycles (26–35 days) and with no history of endometriosis. Endometrial biopsies obtained during the window of implantation timed to the lutenizing hormone surge were compared with biopsies collected during the late proliferative phase (109). Genes found to be differentially expressed by Kao et al. that were also differentially expressed in this study included members of the interleukin family, proline-rich proteins, and genes related to the progesterone receptor.

Oviductal glycoprotein 1 (*OVGP1*), found by Arimoto et al. (102) to be down-regulated in ovarian endometriotic tissue throughout the menstrual cycle, was down-regulated 4.190-fold (p=1.67 x 10-3) in the eutopic endometrium of women with endometriosis in this study. This gene is involved in protecting the early embryo and fallopian tube from noxious extracellular environments. It is secreted from non-ciliated

oviductal epithelial cells and is expressed greatest during ovulation, and decreased expression is speculated to be associated with infertility in women with endometriosis (115). Its decreased expression in the eutopic endometrium of women with endometriosis may play an even greater role in endometriosis-associated infertility, by making the endometrium less capable of protecting an embryo during and after implantation.

RT-PCR Validation of Microarray Studies

The average fold-change of each gene utilized in the validation studies of the eutopic endometrium microarrays corresponded to the array results with regard to up-regulation or down-regulation. However, the magnitude of expression was not always similar. This was especially true in the case of mammaglobin, with an average fold-change of 37.908 via RT-PCR and 4.469 via microarray analysis. *GADD45B*, on the other hand, demonstrated excellent correlation in expression direction and magnitude between the microarray and RT-PCR results, with an average fold-change of 3.133 via the RT-PCR analysis, concurrent with the microarray fold-change of 3.17.

What is the significance of the increase in mammaglobin expression? Could this be a potential marker for endometriotic disease? Despite its dramatic up-regulation in four of the six women with endometriosis (per RT-PCR), it was actually down-regulated via RT-PCR validation in the other two samples. Knowing the phase of the menstrual cycle for each participant would have been helpful in clarifying if this dramatic change in expression was due to endometriosis or to changes in the uterine hormonal environment. Mammaglobin was one of the genes found to be differentially regulated in the study of

Kao et al. comparing gene expression in the window of implantation versus the late proliferative phase (109). It was up-regulated 12.4-fold ($p=0.0255$) during the window of implantation. Mammaglobin is considered to be a breast-specific uteroglobin, and is most noted for its expression in breast cancer. It is also a member of the secretory lipophilin family of proteins prominent in glandular secretions and hormone responsive tissues. Its role in human endometrium during the window of implantation has not been determined (109). It would be interesting to look further into the differential expression of this gene to determine whether its up-regulation is associated with the menstrual cycle phase or whether it plays a role in endometriotic disease. Of note, mammaglobin was not found to be differentially expressed in Kao's subsequent study of differential gene expression in women with endometriosis (107).

In the case of the G2 and S Phase Expressed gene, although the average fold change did show an increase in expression in the endometriosis participants via RT-PCR (1.488), only one of six endometriosis subjects rendered an increase in gene expression (E03), with a fold change of 7.382. The other five subjects actually showed decreased expression versus the normal pool, with all fold changes less than one. When the microarray results for this gene were examined by individuals rather than by the average, it was found that all individuals showed an up-regulation of gene expression. The most likely reason for this discrepancy involves the selection of primers utilized to validate the expression of this gene. An independent primer set was created for this gene utilizing OligoPerfect Designer versus utilizing the sequence on the array. Therefore, the primer set utilized for the RT-PCR validation studies may have amplified a different portion of the gene or an isomer of the gene, instead of the region imprinted on the arrays.

In the same fashion, the *SOCS2* transcript was found by RT-PCR to be in greater abundance in the normal pelvic peritoneum of women with endometriosis versus the normal participant pool (average fold change of 2.240). Microarray analysis had rendered this gene down-regulated at with an average fold change of -2.15. The other two genes transcripts examined in normal pelvic peritoneum, *TNIP2* and *MCM5*, were both found to be in greater abundance in the endometriosis participants via RT-PCR (3.833 and 2.877, respectively), which corresponded to the microarray results of +24.3 and +5.14, respectively.

Future Directions

The primary research direction should be an expansion of the normal pelvic peritoneum studies. No other studies have examined this tissue utilizing microarray analysis. It would be beneficial to study larger samples of normal pelvic peritoneum in women with versus those without endometriosis, as well as to compare peritoneal tissue that is adjacent to endometriotic lesions versus peritoneum in areas where endometriosis is not present within the same patient. A necessity for such a study would be the procurement of a surgical instrument that is able to collect a sufficient quantity of tissue. In most laparoscopies for tubal ligation, only two trocars are placed, one for the camera and one for the instrument that is to either cauterize or place a band around the fallopian tube. The use of only one instrument makes the collection of a tissue biopsy difficult; however, the placement of a third trocar would be extraneous for this surgical procedure. In contrast, the majority of diagnostic laparoscopies have greater than two trocars in

place, which allows for one instrument to grasp the peritoneum and a second instrument for biopsying.

Microarray analysis of peritoneal macrophages in women with and without endometriosis is another area of research in which there has been a lack of publications. For such a study, ideally peritoneal cells should be collected through washings during laparoscopy and the macrophages isolated via negative selection to inflict the least amount of trauma and reduce the likelihood of changes in gene expression. Total RNA isolated from these cells could be hybridized to microarrays specific for inflammatory and cytotoxic pathways to determine if peritoneal macrophages in women with endometriosis demonstrate a decreased ability to remove "foreign" cells from the peritoneal environment. The first studies could be conducted on whole populations of peritoneal cells, with RNA extraction occurring immediately after collection, to determine the gene expression of the peritoneal environment globally. This analysis could be further extrapolated throughout different stages of endometriosis to examine if the characteristics of macrophages change as the disease progresses.

Laser capture microdissection (LCM) is a technique that has allowed microarray analysis of individual cell types from harvested human tissues. It has been utilized in a recent microarray study of deep endometriosis (116). LCM would allow selection for endometrial cells with or without examining stromal cell gene expression as well. This technology is also necessary for examining endometriotic lesions in the peritoneal cavity to ensure that peritoneal or ovarian cells are not included in the analysis.

Recommendations for Similar Studies

Certain elements would enhance the results of a study similar to this one. First of all, access to a larger patient population would enable recruitment at more surgeries and more opportunities for adequate sample collection. In addition, a larger patient population would likely allow for the recruitment of more women with endometriosis of all stages and studies to be performed examining gene expression throughout disease progression.

Depending upon the type of hospital and clinic system, recruitment of participants might be possible prior to the day of surgery, as was the case with this study. If the potential subjects are direct patients of the principal investigator, an introduction to the idea of participation in a research study could be made at the time of surgery scheduling, and a research packet sent home with the patient to look over prior to consideration. This would save time on the day of surgery as well and prevent any delays in the surgery schedule. This also might help on reliability of a patient showing up for their scheduled surgery, which was often an impediment to participant recruitment in this study.

Recruitment during a specific phase of the menstrual cycle might also be able to be coordinated in a research situation where the participants were direct patients of the principal investigator. In the current research study, most of the patients were not utilizing any form of hormonal contraception and/or were not compliant with use. In addition, by approaching participants the day of surgery, it was not possible to schedule or reschedule a participant based upon their menstrual cycle phase. Throughout the analysis of this study's data, alteration of gene expression based upon phase of the menstrual cycle has definitely been a great consideration. As mentioned previously,

several of the genes found to be significantly up- or down-regulated could be due to the presence of endometriotic disease or whether the participants were in the proliferative or secretory phase. Therefore, it would be necessary to determine the menstrual phase of recruited participants and to coordinate collection of samples during the same portion of the cycle.

Limitations to Analysis

Microarrays are an inherently difficult medium with which to work. There are many opportunities for error, particularly in the initial printing of the arrays, as well as in subsequent handling during analysis. One of the greatest difficulties involves proper washing and drying of the arrays following hybridizations. If the slide is allowed to dry, even briefly, throughout the washing process, it can result in residual salts on the array and interfere with scanning. In addition, care must be taken to never touch the array without gloves on to prevent fingerprints and to remove of all dust particles that could render one or more genes unusable. As a result, throughout the image analysis and spot quantification individual genes, and in some instances entire blocks of genes, had to be flagged from analysis due to the presence of background or a dust particle. In addition, some printing errors became evident through scanning, where one spot overlapped with another or alignment of an entire row was "off," and therefore both had to be flagged and removed from analysis.

This study was not able to look at gene expression of the entire genome or sample every endometriosis patient. The arrays utilized allow examination of just over 21,000 of the 30,000 known human genes, and therefore, did not provide a complete examination of

every possible gene that could be involved in pathogenesis of the disease. In addition, the participants enrolled were women from the Tulsa metropolitan area, and within that population, only those whose surgery was conducted at Tulsa Regional Medical Center. Other variables that were not controlled for, including menstrual cycle phase, race, ethnicity, method of hormonal contraception, and body mass index, affect the generalizablility of this study's results.

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APPENDIX I

The following documents are the IRB approval letters from the Oklahoma State University Center for Health Sciences Institutional Review Board.

Oklahoma State University
Center for Health Scineces
College of Osteopathic Medicine

Institutional Review Board

Memo

To: Melanie Y. McKean, D.O./Ph. D. Student
Biochemistry Department

From: Stephen Eddy, D.O., M.P.H.
Chairman, Institutional Review Board

Date: May 1, 2002

Re: Approval of Protocol – IRB # 2002006
Approval of the Informed Consent Contingent Upon Requested Changes

Title: **DNA Microarray Analysis of Endometriosis: A Comparison of Gene Expression in Normal Endometrium versus Four Stages of Endometriotic Lesions**

During the May 2002 meeting The Institutional Review Board of OSU-COM reviewed and approved Protocol – IRB # 2002006. The board also approved the Informed Consent Form contingent upon the following changes:

1) on page 1, under the heading "The Study Design" in the sentence in the second bullet change the word "subjects" to "**participants**"
2) on page 2, at the end of the first open bullet that says, "An endometrial biopsy is a sample of the tissue lining the inside of the uterus." **give a short explanation of how the biopsy will be done**
3) on page 2,in the second bullet change the words "the patient is" to read "**you are**"
4) on page 2, place the word "**Risks:**" above the second paragraph below the bullets that begins "As with any surgical procedure, there are risks..."
5) on page 2, in the third paragraph below the bullets in the sentence that begins, "Small hematomas", change the words "will be" to "**should be**"

Oklahoma State University
College of Osteopathic Medicine

Institutional Review Board

Memo

To: Melanie Y. McKean, D.O./Ph. D. Student
Biochemistry Department

From: Stephen Eddy, D.O.
Chairman, Institutional Review Board

S. Eddy, D.O. expedited approval

Date: May 16, 2002

Re: **Expedited Approval of Informed Consent for Protocol – IRB # 2002006**

Title: **DNA Microarray Analysis of Endometriosis: A Comparison of Gene Expression in Normal Endometrium versus Four Stages Of Endometriotic Lesions**

Under the authority of Title 45, Subtitle A, Part 46, Subpart A (45 CFR 46) and the OSU-COM Institutional Review Board, an expedited review was performed and approval granted for the above-named protocol on May 16, 2002.

This protocol will be submitted to the full Institutional Review Board at the June 5, 2002, meeting. It is the board's prerogative to require additional information or changes in the protocol. You will be informed if any changes are required.

It is your responsibility as principal investigator to promptly report serious adverse events and patient deaths to this IRB. Any revisions or amendments to the approved protocol must be submitted and approved before implementation.

Principal investigators, collaborating investigators, study coordinators and other personnel who have contact with **data or subjects** involved in human research are required to receive **annual training on human subjects**, before beginning a research project. Please contact Steve Phillips at (918) 561-8488 about how you can fulfill these requirements if you have not documented previous training.

You are free to begin the study once all persons involved with your study have completed the above-mentioned training. A stamped "Approved" copy of the Informed Consent is included. Please use it when consenting patients for this study. An annual review for this Protocol will be due before May 1, 2003. If you have any questions please contact: Teri Bycroft at (918) 699-8643.

APPENDIX II

The following documents are the most recent versions of the informed consent letters for both the normal participants and the endometriosis participants.

OKLAHOMA STATE UNIVERSITY COLLEGE OF OSTEOPATHIC MEDICINE

PATIENT INFORMATION AND CONSENT FORM

Title of Project: DNA Microarray Analysis of Endometriosis: A Comparison of Gene Expression in Normal Endometrium versus Four Stages of Endometriotic Lesions

Principal Investigator: Melanie Y. McKean, D.O./Ph.D. Student

Sub-Investigators: Marty Beal, D.O., Sheila Carnett, D.O.

You are being asked to take part in a research study. If this consent form contains any words you do not understand, please ask your doctor or the staff to explain these words so that you understand them. This consent form contains important facts to help you decide if it is in your best interest to take part in this study.

Your participation in this research study is voluntary. You are free to refuse to participate in any procedure and to refuse to answer any question at any time, and are free to withdraw your consent, and to withdraw from the research at any time without penalty.

This research study involves the disease endometriosis. Endometriosis is a common disease among women of reproductive age, and it involves the presence and growth of endometrial tissue (the tissue lining the inside of the uterus that is eliminated each month by a menstrual period) on the outside of uterus. Common symptoms include menstrual cramping, pain during sexual intercourse, and infertility or the inability to become pregnant. Many research studies have shown that endometriosis may have a genetic or heritable basis. A woman with endometriosis often has a mother or sister with endometriosis too. However, endometriosis can also develop in women without a family history of the disease.

One type of cell, the macrophage, may become stimulated or activated in response to foreign materials. Macrophages play an important role in killing foreign substances in the body, and they release substances that stimulate other cells. Women with endometriosis have been found to have more macrophages and more active macrophages in the fluid surrounding the uterus than women without endometriosis. However, the direct effects of these differences have not been determined.

The purpose of this study, therefore, is to gain knowledge regarding how and why endometriosis develops in some women and not others. By examining tissue from women both with and without endometriosis, heritable units or genes can be studied to see if they are active in normal tissue or diseased tissue or both. Those genes active in diseased tissue can then be further studied to determine their specific role in endometriosis development and progression.

The Study Design:

- In this study, we are asking women who are already undergoing surgery to participate.
- For the participants without endometriosis, women undergoing tubal ligation (getting your tubes tied) will be asked to participate.
- Other women who present with pelvic pain and/or infertility, and are planning to undergo surgery, will be asked to participate as a potential endometriosis participant should disease be found when visually inspected by their physician during the surgical procedure.
- If you agree to participate, you will be assigned a number and asked to complete a short medical history form discussing your personal medical history and family history, surgical history, infertility, prescribed and herbal medication use, and pain history. The information linking your name with your study number will be kept in a confidential file during the study that will only be accessed by Dr. Beal or Dr. Carnett. At the completion of this study, this information will be destroyed.

- Three samples will be taken: (1) an endometrial biopsy, (2) a peritoneal biopsy, and (3) peritoneal fluid.
    - An endometrial biopsy is a sample of the tissue lining the inside of the uterus. A thin instrument called a "pipelle" that looks like a narrow drinking straw is inserted through the opening in your cervix and into the uterus. It is pushed forward several inches. A small wire is pulled out of this pipelle once it is inserted. Pulling out the wire makes the pipelle hollow and also creates suction, which draws some of the cells in the lining of your uterus into the pipelle through a hole at its end. Your doctor will move the pipelle forwards and backwards a few times to get a good sample before pulling it out.
    - A peritoneal biopsy is a sample of the tissue lining the inside wall of the abdomen. It is often the site of endometriosis tissue.
    - Peritoneal fluid is fluid found inside of the body around and behind the uterus. It is typically removed during surgeries involving the pelvic region of the body.
- The samples will be taken while you are under anesthesia during the surgery.
- An analysis will then be performed on the tissue samples. Genes, or heritable units, will be
- examined to determine which ones are active in normal tissue, which are active in endometriotic tissue, and how this activity is different in women with endometriosis versus women without endometriosis.

Participation in this study will not cost you anything. The samples will be taken as part of the normal surgical procedure that you are planning to undergo for either tubal ligation or pain evaluation. Refusal to participate in this study WILL NOT affect the timing or care involved in your planned surgical procedure.

Risks:
As with any surgical procedure, there are risks such as **infection, delayed wound healing, hematoma formation (a collection of blood inside the body in and around where the incision is made), bleeding, and possible reactions from anesthesia.** These complications are uncommon. You may also experience some **pain** after the surgical procedure, as your incisions heal. Your physician will explain the amount and type of pain to be experienced.

Infection can result from any surgery and produce swelling, tenderness, pain, and fever. Almost all infections appear within a few days of the operation but on rare occasions may appear at any time after your surgery. Small hematomas should be absorbed by your body, like any bruise, but large ones may have to be drained surgically to permit proper healing. Any incision in the skin will leave a scar. Surgical techniques, under most circumstances, can minimize, though not eliminate, scars.

Benefits:
You will derive no direct benefits from your participation in this study. The indirect benefits of participating in this study may include assisting in an investigation for the genes responsible for the development of a disease that affects 5-15% of normal women and up to 60-80% of women with pelvic pain and/or infertility. This information could lead to more specific treatments, including refined surgical procedures, drug treatments, and other therapies designed for patients suffering from endometriosis.

The records of this study will be kept confidential and you will not be identifiable by name or description in any reports or publications. Reviews may be required for research or monitoring purposes by Dr. Marty Beal, the Oklahoma State University Center for Health Sciences Institutional Review Board or its agents, and other government agencies or as required by law.

NOTICE TO PATIENTS: Information in your medical record that you have a communicable or venereal disease is made confidential by law and cannot be released without your permission except in limited circumstances including release to persons who have risk exposures, release pursuant to an order of the court or the Department of Health, release among health care providers or release for statistical or epidemiological purposes. When such information is released, it cannot contain information from which you could be identified unless release of that identifying information is authorized by you, by an order of the court or the Department of Health or by law.

I understand this authorization is subject to revocation by me at any time except to the extent that action has already been taken in reliance on it. I UNDERSTAND THAT MY MEDICAL RECORDS MAY CONTAIN INFORMATION THAT INDICATES THAT I HAVE A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT IS NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA OR THE HUMAN IMMUNODEFICIENCY VIRUS, ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS). With this knowledge I give my consent to the release of all information in my medical records including any information concerning my identity and release Oklahoma State University-College of Osteopathic Medicine, its agents and employees from any liability in connection with the release of the information relating to a communicable or venereal disease contained therein.

I understand that Oklahoma State University-Center for Health Sciences will not provide compensation and will not provide medical treatment without charge for any injury as a result of and during the course of this research investigation.

By agreeing to participate in this research and signing this form, you do not waive any of your legal rights, nor are the investigators, the institutions, or their agents free from liability or negligence.

If you have any questions about your rights as a study participant, you may contact Dr. Stephen Eddy at Oklahoma State University-Center for Health Sciences, (918) 561-8287.

If you have any questions about this study or research procedures, please contact the principal investigator, Melanie Y. McKean at (918) 852-7270, 1111 W. 17th Street, Tulsa, OK, 74017, at any time (day or night or on the weekends). If you need to report any adverse effects from the research procedures, you will need to contact Dr. Marty Beal, (918) 584-5355, 802 S. Jackson, Suite 420, Tulsa, OK, 74127, at any time.

Voluntary Participation

I have read this consent document or it has been read and clearly explained in the language I understand. My questions have been answered to my satisfaction. I freely consent to participate in this study under the conditions described. I agree to the release of records for review as described under the above conditions. I understand that this consent may be canceled at any time. I have been told of the potential risks and I agree to participate in this research. I will receive a copy of this completed, signed consent form.

_____
Participant's Name (printed)

_____ _____
Participant's Signature Date

_____ _____
Witness's Signature Date

_____ _____
Principal Investigator's Signature Date

OKLAHOMA STATE UNIVERSITY
CENTER FOR HEALTH SCIENCES

AUTHORIZATION TO PERMIT THE USE AND DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES ADDENDUM TO RESEARCH CONSENT FORM

TITLE: DNA Microarray Analysis of Endometriosis: A Comparison of Gene Expression in Normal Endometrium versus Four Stages of Endometriotic Lesions
PRINCIPAL INVESTIGATOR: Melanie Y. McKean, D.O./Ph.D. student
PRINCIPAL INVESTIGATOR'S ADDRESS: 1111 W. 17th Street, Graduate Room 336 Tulsa, OK 74107
PRINCIPAL INVESTIGATOR'S TELEPHONE NUMBER: (918) 852-7270
OTHER INVESTIGATORS: J. Martin Beal, D.O., Sheila Carnett, D.O.

You have given or will give consent to participate in the above-named research study. The purpose of this additional form is to provide you with specific information regarding the use and disclosure of your protected health information, that is information about your health care that has a way that you can be identified with it, for the purpose of this research study. While much of this information was provided to you in the consent form, recently enacted laws, focused on the privacy of medical information, require that this information be addressed in a certain manner. Through the use of this additional form, we are seeking your authorization for the use and disclosure of your medical information for the purpose of this research study as required in these recently enacted laws.

This research study will involve the recording of current and/or future protected health information from your hospital and/or physician office records. The information that will be recorded will be limited to information concerning the stage of endometriosis that was observed during the laparoscopic surgery (Stage I, II, III, or IV) by the physician. This information will be used for the purpose of categorizing the samples obtained during the surgery.

Authorized representatives of the Institutional Review Board that reviewed and approved the performance of this study may review your protected health information for the purpose of monitoring the appropriate conduct of this research study.

Staff of your hospital, the OSU-CHS clinics or other affiliated health care providers will have access to your protected health information as needed for the purpose of (1) following treatment orders made by the investigators for health care services (e.g., laboratory tests, diagnostic procedures) related to your participation in the research study; (2) billing for tests and procedures ordered by the investigators; and (3) for internal hospital operations (i.e. quality assurance).

In unusual cases, the investigators may be required to release your protected research information in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform the appropriate agencies as required by Oklahoma law.

Although the researchers may report their findings about this research study in scientific journals or meetings, you will not be identified in their reports or articles.

The researchers, OSU-CHS, and other health care providers affiliated with OSU-CHS will try to keep all information about your participation in this research study confidential, but absolute confidentiality cannot be guaranteed.

In accordance with the OSU-CHS Notice of Privacy Practices document which you have been provided, or will be provided when you present for services at the OSU Clinic, OSU Physicians Building, or Tulsa Regional Medical Center, you are permitted to see the information contained in your medical records and kept by your health care provider unless otherwise specifically stated below.

Your authorization (permission) to use and disclose your protected health information for the purpose of this research study is completely voluntary. However, if you do not provide your written authorization for the use and disclosure of your protected health information, you will not be allowed to participate or continue to participate in the research study.

Whether or not you provide your authorization for the research use and disclosure of your medical information will have no affect on your current or future medical care at the OSU-CHS-affiliated clinic or hospitals where you receive care or your current or future relationship with a health insurance carrier. Whether or not you provide this authorization will have no affect on your relationship with OSU-CHS.

You may withdraw, at any time, your authorization for the use and disclosure of your protected health information for the purpose of this research study. However, if you withdraw your authorization for the use and disclosure of your protected health information, you will also be withdrawn from further participation in this research study. Any protected health information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your authorization may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your authorization you should notify the principal investigator of this research study. Contact information is listed on the first page of this form. The investigator will ask you to sign a document with the date you asked to withdraw.

Your decision to withdraw your authorization for the research use and disclosure of your medical information will have no affect on your current or future medical care at the OSU-CHS-affiliated clinic or hospitals or your current or future relationship with a health insurance provider. Your decision to withdraw this authorization will have no affect on your current or future relationship with the OSU-CHS.

The investigators may continue to use and disclose your protected health information for the purposed described above for an indefinite period of time.

Voluntary Consent

All of the above has been explained to me and all of my current questions have been answered. Throughout my participation in this research study, I have been encouraged to ask any additional questions I may have about the research use and disclosure of my protected health information. Such future questions will be answered by the investigators listed on the first page of this form.

Any questions I have about my rights associated with the research use and disclosure of my medical information will be answered by Teri Bycroft at (918) 699-8643.

By signing this form, I agree to allow the use and disclosure of my medical information for the purposes described above. With this knowledge, I give my authorization and consent to the use and disclosure as described in this document to the people identified in this form. A copy of this authorization form will be given to me.

_____ _____

Participant's Signature Date

OKLAHOMA STATE UNIVERSITY COLLEGE OF OSTEOPATHIC MEDICINE

PATIENT INFORMATION AND CONSENT FORM

Title of Project: DNA Microarray Analysis of Endometriosis: A Comparison of Gene Expression in Normal Endometrium versus Four Stages of Endometriotic Lesions

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The Study Design:

- In this study, we are asking women who are already undergoing surgery to participate.
- For the participants without endometriosis, women undergoing tubal ligation (getting your tubes tied) will be asked to participate.
- Other women who present with pelvic pain and/or infertility, and are planning to undergo surgery, will be asked to participate as a potential endometriosis participant should disease be found when visually inspected by their physician during the surgical procedure.
- If you agree to participate, you will be assigned a number and asked to complete a short medical history form discussing your personal medical history and family history, surgical history, infertility, prescribed and herbal medication use, and pain history. The information linking your name with your study number will be kept in a confidential file during the study that will only be accessed by Dr. Beal or Dr. Carnett. At the completion of this study, this information will be destroyed.

- Five samples will be taken: (1) a blood sample, (2) an endometrial biopsy, (3) a peritoneal biopsy of normal pelvic peritoneum, (4) a peritoneal biopsy of endometriotic tissue, and (5) peritoneal fluid.
    - The blood sample collected will be approximately 2-3 tablespoons.
    - An endometrial biopsy is a sample of the tissue lining the inside of the uterus. A thin instrument called a "pipelle" that looks like a narrow drinking straw is inserted through the opening in your cervix and into the uterus. It is pushed forward several inches. A small wire is pulled out of this pipelle once it is inserted. Pulling out the wire makes the pipelle hollow and also creates suction, which draws some of the cells in the lining of your uterus into the pipelle through a hole at its end. Your doctor will move the pipelle forwards and backwards a few times to get a good sample before pulling it out.
    - A peritoneal biopsy is a sample of the tissue lining the inside wall of the abdomen. It is often the site of endometriosis tissue. One biopsy will be taken from normal pelvic peritoneum. A second biopsy will be taken from endometriotic tissue.
    - Peritoneal fluid is fluid found inside of the body around and behind the uterus. It is typically removed during surgeries involving the pelvic region of the body.
- All biopsies will be taken while you are under anesthesia during the surgery.
- An analysis will then be performed on the tissue samples. Genes, or heritable units, will be examined to determine which ones are active in normal tissue, which are active in endometriotic tissue, and how this activity is different in women with endometriosis versus women without endometriosis.

Participation in this study will not cost you anything. The samples will be taken as part of the normal surgical procedure that you are planning to undergo for either tubal ligation or pain evaluation. Refusal to participate in this study WILL NOT affect the timing or care involved in your planned surgical procedure.

Risks:
As with any surgical procedure, there are risks such as **infection, delayed wound healing, hematoma formation (a collection of blood inside the body in and around where the incision is made), bleeding, and possible reactions from anesthesia.** These complications are uncommon. You may also experience some **pain** after the surgical procedure, as your incisions heal. Your physician will explain the amount and type of pain to be experienced.

Infection can result from any surgery and produce swelling, tenderness, pain, and fever. Almost all infections appear within a few days of the operation but on rare occasions may appear at any time after your surgery. Small hematomas should be absorbed by your body, like any bruise, but large ones may have to be drained surgically to permit proper healing. Any incision in the skin will leave a scar. Surgical techniques, under most circumstances, can minimize, though not eliminate, scars.

The risks from obtaining a blood sample include bruising, or small hematoma formation, as well as tenderness or pain.

Benefits:
You will derive no direct benefits from your participation in this study. The indirect benefits of participating in this study may include assisting in an investigation for the genes responsible for the development of a disease that affects 5-15% of normal women and up to 60-80% of women with pelvic pain and/or infertility. This information could lead to more specific treatments, including refined surgical procedures, drug treatments, and other therapies designed for patients suffering from endometriosis.

The records of this study will be kept confidential and you will not be identifiable by name or description in any reports or publications. Reviews may be required for research or monitoring purposes by Dr. Marty Beal, the Oklahoma State University Center for Health Sciences Institutional Review Board or its agents, and other government agencies or as required by law.

NOTICE TO PATIENTS: Information in your medical record that you have a communicable or venereal disease is made confidential by law and cannot be released without your permission except in limited circumstances including release to persons who have risk exposures, release pursuant to an order of the court or the Department of Health, release among health care providers or release for statistical or epidemiological purposes. When such information is released, it cannot contain information from which you could be identified unless release of that identifying information is authorized by you, by an order of the court or the Department of Health or by law.

I understand this authorization is subject to revocation by me at any time except to the extent that action has already been taken in reliance on it. I UNDERSTAND THAT MY MEDICAL RECORDS MAY CONTAIN INFORMATION THAT INDICATES THAT I HAVE A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT IS NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA OR THE HUMAN IMMUNODEFICIENCY VIRUS, ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS). With this knowledge I give my consent to the release of all information in my medical records including any information concerning my identity and release Oklahoma State University-College of Osteopathic Medicine, its agents and employees from any liability in connection with the release of the information relating to a communicable or venereal disease contained therein.

I understand that Oklahoma State University-Center for Health Sciences will not provide compensation and will not provide medical treatment without charge for any injury as a result of and during the course of this research investigation.

By agreeing to participate in this research and signing this form, you do not waive any of your legal rights, nor are the investigators, the institutions, or their agents free from liability or negligence.

If you have any questions about your rights as a study participant, you may contact Dr. Stephen Eddy at Oklahoma State University-Center for Health Sciences, (918) 561-8287.

If you have any questions about this study or research procedures, please contact the principal investigator, Melanie Y. McKean at (918) 852-7270, 1111 W. 17th Street, Tulsa, OK, 74017, at any time (day or night or on the weekends). If you need to report any adverse effects from the research procedures, you will need to contact Dr. Marty Beal, (918) 584-5355, 802 S. Jackson, Suite 420, Tulsa, OK, 74127, at any time.

Voluntary Participation

I have read this consent document or it has been read and clearly explained in the language I understand. My questions have been answered to my satisfaction. I freely consent to participate in this study under the conditions described. I agree to the release of records for review as described under the above conditions. I understand that this consent may be canceled at any time. I have been told of the potential risks and I agree to participate in this research. I will receive a copy of this completed, signed consent form.

_____
Participant's Name (printed)

_____ _____
Participant's Signature Date

_____ _____
Witness's Signature Date

_____ _____
Principal Investigator's Signature Date

OKLAHOMA STATE UNIVERSITY
CENTER FOR HEALTH SCIENCES

AUTHORIZATION TO PERMIT THE USE AND DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES ADDENDUM TO RESEARCH CONSENT FORM

TITLE: DNA Microarray Analysis of Endometriosis: A Comparison of Gene Expression in Normal Endometrium versus Four Stages of Endometriotic Lesions
PRINCIPAL INVESTIGATOR: Melanie Y. McKean, D.O./Ph.D. student
PRINCIPAL INVESTIGATOR'S ADDRESS: 1111 W. 17th Street, Graduate Room 336 Tulsa, OK 74107
PRINCIPAL INVESTIGATOR'S TELEPHONE NUMBER: (918) 852-7270
OTHER INVESTIGATORS: J. Martin Beal, D.O., Sheila Carnett, D.O.

You have given or will give consent to participate in the above-named research study. The purpose of this additional form is to provide you with specific information regarding the use and disclosure of your protected health information, that is information about your health care that has a way that you can be identified with it, for the purpose of this research study. While much of this information was provided to you in the consent form, recently enacted laws, focused on the privacy of medical information, require that this information be addressed in a certain manner. Through the use of this additional form, we are seeking your authorization for the use and disclosure of your medical information for the purpose of this research study as required in these recently enacted laws.

This research study will involve the recording of current and/or future protected health information from your hospital and/or physician office records. The information that will be recorded will be limited to information concerning the stage of endometriosis that was observed during the laparoscopic surgery (Stage I, II, III, or IV) by the physician. This information will be used for the purpose of categorizing the samples obtained during the surgery.

Authorized representatives of the Institutional Review Board that reviewed and approved the performance of this study may review your protected health information for the purpose of monitoring the appropriate conduct of this research study.

Staff of your hospital, the OSU-CHS clinics or other affiliated health care providers will have access to your protected health information as needed for the purpose of (1) following treatment orders made by the investigators for health care services (e.g., laboratory tests, diagnostic procedures) related to your participation in the research study; (2) billing for tests and procedures ordered by the investigators; and (3) for internal hospital operations (i.e. quality assurance).

In unusual cases, the investigators may be required to release your protected research information in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform the appropriate agencies as required by Oklahoma law.

Although the researchers may report their findings about this research study in scientific journals or meetings, you will not be identified in their reports or articles.

The researchers, OSU-CHS, and other health care providers affiliated with OSU-CHS will try to keep all information about your participation in this research study confidential, but absolute confidentiality cannot be guaranteed.

In accordance with the OSU-CHS Notice of Privacy Practices document which you have been provided, or will be provided when you present for services at the OSU Clinic, OSU Physicians Building, or Tulsa Regional Medical Center, you are permitted to see the information contained in your medical records and kept by your health care provider unless otherwise specifically stated below.

Your authorization (permission) to use and disclose your protected health information for the purpose of this research study is completely voluntary. However, if you do not provide your written authorization for the use and disclosure of your protected health information, you will not be allowed to participate or continue to participate in the research study.

Whether or not you provide your authorization for the research use and disclosure of your medical information will have no affect on your current or future medical care at the OSU-CHS-affiliated clinic or hospitals where you receive care or your current or future relationship with a health insurance carrier. Whether or not you provide this authorization will have no affect on your relationship with OSU-CHS.

You may withdraw, at any time, your authorization for the use and disclosure of your protected health information for the purpose of this research study. However, if you withdraw your authorization for the use and disclosure of your protected health information, you will also be withdrawn from further participation in this research study. Any protected health information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your authorization may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your authorization you should notify the principal investigator of this research study. Contact information is listed on the first page of this form. The investigator will ask you to sign a document with the date you asked to withdraw.

Your decision to withdraw your authorization for the research use and disclosure of your medical information will have no affect on your current or future medical care at the OSU-CHS-affiliated clinic or hospitals or your current or future relationship with a health insurance provider. Your decision to withdraw this authorization will have no affect on your current or future relationship with the OSU-CHS.

The investigators may continue to use and disclose your protected health information for the purposed described above for an indefinite period of time.

Voluntary Consent

All of the above has been explained to me and all of my current questions have been answered. Throughout my participation in this research study, I have been encouraged to ask any additional questions I may have about the research use and disclosure of my protected health information. Such future questions will be answered by the investigators listed on the first page of this form.

Any questions I have about my rights associated with the research use and disclosure of my medical information will be answered by Teri Bycroft at (918) 699-8643.

By signing this form, I agree to allow the use and disclosure of my medical information for the purposes described above. With this knowledge, I give my authorization and consent to the use and disclosure as described in this document to the people identified in this form. A copy of this authorization form will be given to me.

_____ _____

Participant's Signature Date

VITA

Melanie Ann Yuracko McKean

Candidate for the Degree of

Doctor of Philosophy

Thesis: DIFFERENTIAL GENE EXPRESSION OF EUTOPIC ENDOMETRIUM AND NORMAL PELVIC PERITONEUM IN WOMEN WITH AND WITHOUT ENDOMETRIOSIS

Major Field: Biomedical Science

Biographical:

Personal Data: Born in St. Clair, Missouri, May 22, 1977, the daughter of Betty V. and John D. Yuracko. Married to Christopher James McKean on July 8, 2000. Expecting first child in May, 2006.

Education: Received a Bachelor of Science in Biology and a minor in Women's Studies from Truman State University, Kirksville, MO, in 1999; Doctor of Osteopathy degree to be conferred at Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma in May, 2006. Completed the requirements for the Doctor of Philosophy degree at Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma in May, 2006.

Professional Memberships: Alpha Phi International Fraternity Alumnae Association, American Medical Women's Association, American Osteopathic Association, Student Osteopathic Medical Association, Sigma Sigma Phi National Osteopathic Service Honor Society, Oklahoma Osteopathic Association, American College of Physicians, American Psychiatric Association.

Name: Melanie Ann Yuracko McKean Date of Degree: May 2006

Institution: Oklahoma State University Center for Health Sciences

Location: Tulsa, Oklahoma

Title of Study: DIFFERENTIAL GENE EXPRESSION OF EUTOPIC ENDOMETRIUM AND NORMAL PELVIC PERITONEUM IN WOMEN WITH AND WITHOUT ENDOMETRIOSIS

Pages in Study: 165 Candidate for the Degree of Doctor of Philosophy

Major Field: Biomedical Science

Scope and Method of Study: To investigate differential gene expression in women with and without endometriosis. Women already undergoing laparoscopic surgery were recruited as normal and potential endometriosis participants. The normal participants were those women undergoing laparoscopic bilateral tubal ligation, and the endometriosis participants were those undergoing diagnostic laparoscopy to evaluate chronic pelvic pain. Sample collection included an endometrial aspiration, normal pelvic peritoneum, peritoneal fluid, and endometriotic lesion (in those with endometriosis). Total RNA was extracted from tissue samples and put through one round of amplification in the normal pelvic peritoneum studies. Human oligo microarrays with over 21,000 genes were utilized in the analysis. Pooled RNA from normal participants was hybridized against RNA from each endometriosis participant. Microarray analysis was performed utilizing GenePix Pro and GenePix Auto Processor. Microarray results were validated utilizing RT-PCR.

Findings and Conclusions: Seven women with and seven women without endometriosis were utilized in the microarray studies. In the comparison of gene expression in eutopic endometrium, 756 genes were found to be significantly up-regulated or down-regulated in the women with endometriosis versus a normal pool. KEGG pathway analysis revealed genes involved in the following pathways: cell communication, MAPK signaling, cytokine-cytokine receptor interaction, cell cycle, TGF-beta signaling, focal adhesion, cell adhesion, and ECM-receptor interaction. In the comparison of gene expression in normal pelvic peritoneum, 202 genes were found to be significantly up-regulated or down-regulated in women with endometriosis versus those without. These genes are involved in the following pathways: cytokine-cytokine receptor, cell cycle, focal adhesion, regulation of actin cytoskeleton, and leukocyte transendothelial migration.

ADVISER'S APPROVAL: Nancy J. Carpenter, Ph.D.