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Research Article

The Potential of Microarray Databases to Identify Tissue Specific Genes

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Abstract

Tissue specific genes play important roles in development and metabolism. Currently, GenBank has 3363628 GEO Profiles and 397988 microarray data related to various tissues. To evaluate the huge amounts of data and identify tissue specific genes, it is necessary to develop and use new strategies. To that end, this study discusses if microarray and microarray related GEO Profiles are a useful tool to identify new tissue specific genes. In the current study, adipose tissue sellected as a target tissue in order to find new tissue specific genes. Therefore, the human and mouse microarray data were analyzed comparatively. To support the microarray data, adipose tissue related GEO Profiles were selected from PubMed. Subsequently, adipose tissue related microarray and GEO Profiles were analysed simultaneously. According to analysis of microarray and GEO Profiles, Chrdl1 (Chordin-like 1) gene was hypothesized as a novel adipose specific gene. In order to test the hypothesis, RT-PCR analysis were performed for the bovine tissue distribution. As a result, the hypothesis was successfully tested and Chrdl1 gene was found highly specific in bovine adipose tissue than in various other tissues. Thus, it is concluded that microarray and microarray related GEO Profiles are a useful tool to identify new tissue specific genes.

Keywords: *Adipose tissue, Adipose specific gene, Chordin-like 1, Microarray*

Mikroarray Veritabanlarının Doku Spesifik Genlerin Belirlenmesindeki Potansiyeli

Özet

Doku spesifik genler gelişim ve metabolizma da önemli roller oynamaktadır. Günümüzde, GenBankasında farklı dokularla ilgili 3363628 gen ekspresyon profili ve 397988 mikroarray verisi bulunmaktadır. Büyük miktardaki verileri değerlendirmek ve doku spesifik genleri ortaya çıkarabilmek için yeni stratejilerin geliştirilmesi ve kullanılması gerekmektedir. Bu amaçla, bu çalışma mikroarray ve mikroarraylerle ilişkili gen ekspresyon profillerinin yeni doku spesifik genlerin belirlenmesinde kullanışlı bir araç olup olmadığını tartışmaktadır. Bu çalışmada, adipoz doku yeni doku spesifik genlerin belirlenmesi için hedef doku olarak seçilmiştir. Bu amaçla, insan ve fare mikroarray verileri karşılaştırmalı olarak analiz edilmiştir. Mikroarray verilerini desteklemek için, adipoz dokuyla ilgili gen ekspresyon profilleri GenBankasından seçilmiştir. Daha sonra, adipoz dokuyla ilgili mikroarray ve gen ekspresyon profil verileri eş zamanlı olarak değerlendirilmiştir. Mikroarray ve mikroarraylerle ilişkili gen ekspresyon profillerinin analiz sonuçlarına göre, Chrdl1 (Chordin-like 1) geninin yeni bir adipoz spesifik gen olduğuyla ilgili hipotez kurulmuştur. Hipotezi test etmek için, sığır dokularında RT-PCR analizleri gerçekleştirilmiştir. Sonuç olarak, hipotez başarılı bir şekilde test edilmiş ve Chrdl1 geni sığır adipoz dokusunda diğer dokulara göre yüksek derecede spesifik bulunmuştur. Böylelikle, mikroarray ve mikroarraylerle ilişkili gen ekspresyon profillerinin yeni doku spesifik genlerin belirlenmesinde kullanışlı bir araç olduğu sonucuna varılmıştır.

Anahtar sözcükler: *Adipoz doku, Adipoz spesifik gen, Chordin-like 1, Mikroarray*

INTRODUCTION

Since invented in 1995, microarray technologies have been widely used to compare the expression profiles of thousands of genes simultaneously under different biological conditions [1]. Nowadays, high throughput microarray technologies have numerous applications, including the study of gene expression, genotyping, proteomics, cell biology, recognizing infectious diseases, cancer biology, pharmaco genomics, detecting the existence of a pathogen in food samples and identification of microbial isolates [2].

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Obesity is a result of excess adipose development. There is a growing recognatiton that obesity is a metabolic disorder and can cause serious health problems such as heart disease, insulin resistance, hyperglycemia, dyslipidemia, hypertension, obstructive sleep apnea, and certain types of cancer [3,4]. Thus, obesity is approved as one of the most serious public health concerns in the 21st century [5].

Adipose development is significantly influenced by adipose specific genes [6]. To date, approximately 20 genes have been identified that are highly expressed in adipose tissue [7] It is well known that there have been strong relationship between adipose specific genes and obesity both human and animals. One of the most important adipose specific genes leptin and adiponectin plasma levels are decreased in both obese humans and animals [8,9]. Therefore, discoveries of new adipose specific genes will be valuable to the understanding of genetic mechanisms underlying adipose development and obesity and will also contribute to improved animal production by reducing fat accretion. In order to clarify adipose development, it is necessary to develop new methods for finding and understanding the functions of adipose specific genes.

Currently, GenBank has 1674749 GEO Profiles and 19928 microarray data related to adipose tissue [10]. These huge amounts of data are important resource to identify adipose specific genes. Thus, the main objective of this study was elucidate microarray and microarray related GEO Profiles can be used to identify new adipose specific genes. Therefore, human and mouse microarray data were analyzed comparatively. The information from GEO Profiles and literature were also used to support microarray analysis. According to the analysis, Chrdl1 was identified as a novel adipose specific candidate gene. Then, RT-PCR experiments were performed in bovine tissue distribution. The RT - PCR analysis confirmed that Chrdl1 gene was highly specific in bovine adipose tissue. As a result, this study proved that comparative analysis of microarray and microarray related GEO Profiles are very effective tool to identify new tissue specific genes.

MATERIAL and METHODS

Data Mining

To investigate the adipose specific genes, the keywords (adipose tissue) and (adipocyte) were used to search gene expression omnibus (GEO) database from PubMed [10]. Therefore, 1674749 GEO Profiles analysed in (GEO) database from PubMed [10]. Due to tissue distribution similarity GDS3142 various tissues *(Mus musculus)* and GDS596 Large-scale analysis of the human transcriptome (HG-U133A) *(Homo sapiens)* microarray data were selected in GEO database [10]. The GDS3142 [11] microarray consists of 22 different tissues from 10 to 12 wk old C57BL/6 mice and the GDS596 [12] microarray includes 79 physiologically normal tissues obtained from various sources. The dataset file of each microarray was downloaded from GEO database [10]. The GDS3142 [11] dataset file is composed of gene expression profiles. Each tissue except for muscle has 3 biological replicates. Muscle has 4 biological replicates of gene expression profiles. Tissue biological replicates of spleen (GSM252067, GSM252068, GSM252069), muscle (GSM252070, GSM252071, GSM252072, GSM252073), adipose (GSM252093, GSM252094, GSM252095), heart (GSM252113, GSM252114, GSM252115), lung (GSM252080, GSM252081, GSM252082), liver (GSM252074, GSM252075, GSM252076) and kidney (GSM252083, GSM252084, GSM252085) were collected from the GDS3142 [11]. A new Excel file was formulated for the dataset obtained from the GDS3142 [11]. Next, triplicate data from the various tissues were averaged. To find the adipose specific genes, the adipose tissue gene expression value was divided by the various tissue gene expression values. Then, values were averaged again in order to elucidate the common ratio of the various tissues. One common ratio was found which represents the ratio of various tissue expression values versus to adipose tissue. Finally, this ratio was sorted smallest to largest to identify the adipose specific genes.

The GDS596 [12] dataset file also includes gene expression profiles. Each tissue has 2 biological replicates from various sources. Heart (GSM18951, GSM18952), lung (GSM18949, GSM18950), liver (GSM18953, GSM18954) adipocyte (GSM18975, GSM18976), skeletal muscle (GSM19013, GSM19014), and kidney (GSM18955, GSM18956) gene expression profiles were selected from the GDS596 dataset [12]. All of the procedures were repeated for the GDS596 dataset as were used for GDS3142 and the common ratio of the various tissue expression values according to adipocyte were detected. The flow chart of the study illustrated in the schematic diagram shown in *Fig. 1*. To support and predict reliability of microarray data, different GEO profiles were selected from GEO database [10].

Tissue Collection

Adipose tissue, heart, muscle, spleen, lung, liver, and kidney of cattle (Angus; n = 5) were harvested after slaughter of animals at Yılet Meat Company located in Konya province. In this procedure, different equipments are used for each tissue to prevent contamination during the removal of the tissues. Finally, 300 mg tissues put into eppendorf tubes and snap frozen in liquid nitrogen.

RNA Isolation and RT-PCR Detection of Total Gene Expression

To prepare snap frozen animal tissues for RNA extraction, tissues are ground into fine powder to obtain a high yield of RNA by using a tissue homogenizer. Total RNA from the adipose tissue, heart, muscle, spleen, lung, liver, and kidney

of cattle were isolated using GF-1 Total RNA extraction kit (Vivantis) following the manufacturer's instructions. RNA quality was assessed by agarose gel electrophoresis. To make cDNA for various tissues, RT-PCR was performed using 1 µg of total RNA and M-MLV reverse transcriptase (Moloney murine leukemia virus RT, Vivantis). Gene expression was quantified using SYBR Green RT-PCR. The RT-PCR was performed using Quantitect SYBR PCR Master Mix (Qiagen), and SYBR green was used as the detection dye. The Cyclophilin gene was used as a housekeeping gene for normalization of RT-PCR calculation. Chrdl1 gene mRNA expression was normalized to Cyclophilin mRNA. The forward and reverse primers for Cyclophilin (BC102462) were 5`- TTCCATCGTGTGATCAAGGA -3`; 5`- TTAAGCTTGAAGTTCTCATCGG -3`, respectively. The forward and reverse primers for Chrdl1 (540275) were 5`- ACT CCATCACTTCAAGCTGGTGA -3`; 5`- TGCAGTCCAGCT GCAGCTT -3`, respectively. Reactions were performed in duplicate 25µL volumes on Fluorion Real-Time PCR Instrument. Conditions for RT-PCR were 95°C for 15 min, and then 50 cycles of 95°C for 30 s, 60°C for 35 s, and 72°C for 40 s. Finally, Melting curve step was performed in RT-PCR. Relative expression was calculated using the comparative ΔΔCt method for relative quantification [13].

Statistical Analysis

Comparisons of gene expression in various tissues of the mouse and human microarray were performed by one-way ANOVA, respectively. If the P-value was <0.05 in the ANOVA, Duncan multiple range test was performed. All statistical analyses were performed using GLM procedure of SAS statistical software [14].

RESULTS

In this study, statistical analysis revealed that Chrdl1, FABP4 (Fatty Acid Binding Protein 4) and ATGL (Adipose Triglyceride Lipase) genes are highly expressed in human adipocyte and mouse adipose tissue than various tissues as shown *Table 1* and *Table 2* (P<0.0001). Since, there has not been any study that showed adipose specific gene expression of Chrdl1, Chrdl1 gene was selected for further investigation. Therefore, we hypothesize that Chrdl1 gene expression is highly specific in adipose tissue than other tissues. We supported our hypothesis with further information collecting from different GEO profiles. Moreover, our hypothesis was also successfully tested by RT-PCR experiment. To test the hypothesis, RT-PCR performed for bovine tissue distribution. Results demonstrate that Chrdl1 is more highly expressed in bovine adipose tissue than in other tissues (P<0.05) as shown in *Fig. 2*. Considering the literature, Chrdl1 shows a broad expression pattern and functions in many tissues [15-19]. However, our study which represents to our knowledge

Table 1. *Gene expressions of Chrdl1, FABP4 and ATGL in tissue distributions of mouse microarray*
Tablo 1. *Chrdl1, FABP4 ve ATGL genlerinin fare mikroarrayinin doku dağılımındaki gen ekspresyonları*

Gene	Adipose	Muscle	Heart	Lung	Liver	Kidney	Spleen	P Value
FABP4	2924.9±443.9^{a}	198.6±5.9^{b}	296.9±34.9^{b}	86.1±7.7^{b}	82.3±0.9^{b}	118.0±16.0^{b}	74.7±5.6^{b}	P<0.0001
ATGL	4526.4±388.3^{a}	785.7±82.6^{bc}	1165.2±78.8^{b}	453.9±36.5^{bc}	327.2±37.4^{c}	410.2±34.4^{c}	195.8±8.9^{c}	P<0.0001
CHRDL1	825.2±129.8^{a}	99.8±6.9^{b}	76.7±2.0^{b}	110.2±6.8^{b}	64.9±1.2^{b}	97.8±12.0^{b}	92.9±3.4^{b}	P<0.0001
GSM NO	GSM252093	GSM252070	GSM252113	GSM252080	GSM252074	GSM252083	GSM252067	
	GSM252094	GSM252071	GSM252114	GSM252081	GSM252075	GSM252084	GSM252068	
	GSM252095	GSM252072	GSM252115	GSM252081	GSM252076	GSM252085	GSM252069	
		GSM252073						

GSM NO represents the biological replicates of adipose, muscle, heart, lung, liver, spleen and kidney tissues. Biological replicates number for adipose, heart, lung, liver, spleen and kidney tissues (n=3) and muscle tissue (n=4). Results are shown as average ± standard error. Different letters in the same row show significant differences between the averages (P<0.05)

Table 2. *Gene expressions of Chrdl1, FABP4 and ATGL in tissue distributions of human microarray*
Tablo 2. *Chrdl1, FABP4 ve ATGL genlerinin insan mikroarrayinin doku dağılımındaki gen ekspresyonları*

Gene	Adipocyte	Muscle	Heart	Lung	Liver	Kidney	P Value
FABP4	13364400±503100^{a}	446400±130800^{bc}	317400±153700^{bc}	1099850±275350^{b}	123700±74000^{c}	189500±58100^{bc}	P<0.0001
ATGL	2838150±111150^{a}	367800±92800^{c}	853150±260250^{b}	274250±54750^{c}	299600±105500^{c}	357850±76950^{c}	P<0.0001
CHRDL1	1702350±86950^{a}	303450±191450^{b}	130900±80600^{b}	315400±41500^{b}	58550±27050^{b}	240550±24550^{b}	P<0.0001
GSM NO	GSM18975	GSM19013	GSM18951	GSM18949	GSM18953	GSM18955	
	GSM18976	GSM19014	GSM18952	GSM18950	GSM18954	GSM18956	

GSM NO represents the biological replicates of adipocyte, muscle, heart, lung, liver and kidney tissues. Biological replicates number for adipocyte, muscle, heart, lung, liver and kidney tissues (n=2). Results are shown as average ± standard error. Different letters in the same row show significant differences between the averages (P<0.05)

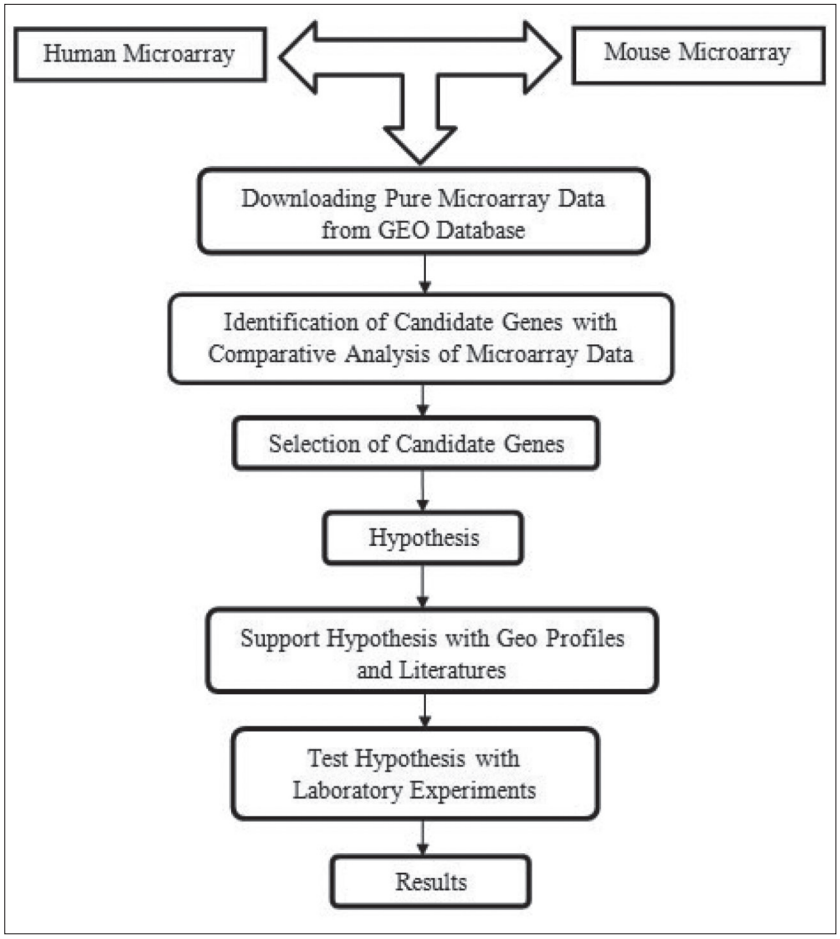

Fig 1. The flow chart of the study

Şekil 1. Çalışmanın akış şeması

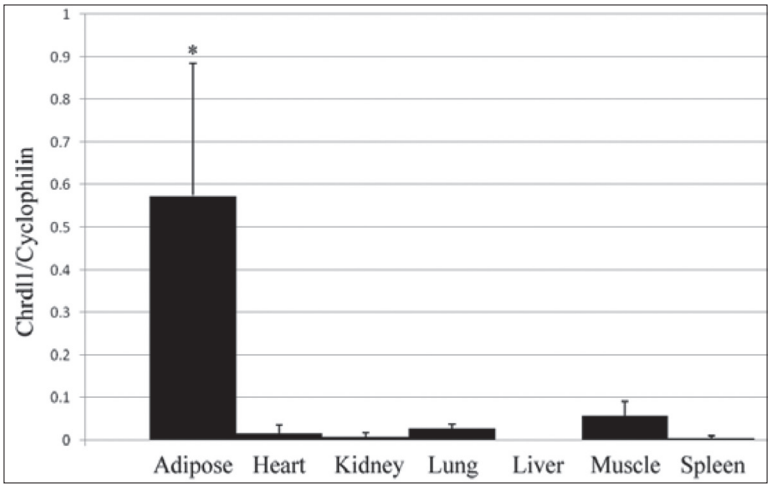

Fig 2. Chrdl1 gene expression in bovine tissue distribution . The y axis shows the relative expresion of Chrdl1\Cyclophilin. The bar graphs represent average ± standard error. (*) sign is shown the gene expression of Chrdl1 in bovine adipose tissue statistically important than other tissues according to the results of one-way ANOVA followed by Duncan multiple range test (P<0.05)

Şekil 2. Sığır doku dağılımında Chrdl1 gen ekpresyonu. Y ekseni Chrdl1\Cyclophilin genlerinin karşılaştırmalı gen ekspresyonunu göstermektedir. Grafikte görülen barlar ortalama ± standart hatayı temsil etmektedir. (*) tek yönlü varyans analizini takiben yapılan Duncan çoklu karşılaştırma testinin sonuçlarına göre Chrdl1 geninin adipoz dokudaki ekspresyonunun diğer dokulara göre istatistiksel olarak önemli olduğunu göstermektedir (P<0.05)

the first report that Chrdl1 gene expression is highly specific in adipose tissue versus other tissues.

DISCUSSION

Currently, microarray technology is used extensively for scientific research. This technology allows screening of thousands of genes simultaneously under different biological conditions. However, the challenging part is how to select the target genes from thousands of genes. As aforementioned, we identified that Chrdl1, FABP4 and ATGL genes are highly specific in adipose tissue by comparative analysis of microarray database. Among these genes, FABP4 and ATGL genes are well known adipose specific genes [20,21]. According to analysis, the FABP4 and ATGL genes were more highly expressed in mouse adipose tissue than in other tissues in the mouse microarray data (P<0.0001) as shown *(Table 1)*. Similarly, the FABP4 and ATGL genes were predominantly expressed in human adipocytes as compared to other tissues in the human

microarray data (P<0.0001) as shown *(Table 2)*. The FABP4 and ATGL genes were selected as examples for how to we analyzed microarray and microarray related GEO Profiles related to adipose tissue.

Fatty Acid Binding Protein 4 (FABP4)

Searching for FABP4 in the GEO Profiles revealed 5788 microarray analyses that contained the FABP4 gene and searching for "adipocyte" as a key word in the GEO Datasets showed 3919 microarray analyses dealing with adipocytes under different developmental, hormonal, nutritional, genetic, and pathological conditions [10]. As shown in GEO Profiles obtained from PubMed [10], GDS2659 demonstrated that FABP4 gene expression was increased during adipocyte differentiation [22]. Li et al.[20] reported that FABP4 was highly expressed during 3T3-L1 adipocyte cell differentiation. This finding was also verified by previous studies [23-25]. The GDS2818 data demonstrated that adipocytes had higher levels of FABP4 gene expression than do preadipocytes [26]. It`s previously reported that FABP4 was more highly expressed in adipocytes than in preadipocytes [27,28]. GDS3688 showed that obese children had higher FABP4 gene expression in adipose tissue [29]. Ma et al.[30] demonstrated that the FABP4 and PPAR-γ genes were more highly expressed in obesity groups than in obesity resistant groups. GDS734 [31] clarified the PPAR-γ induced expression of FABP4 in adipocytes which was confirmed the previous finding of Shin et al.[32] and suggested that PPAR-γ binds to the FABP4 promoter and activates transcription of FABP4. In addition, PPAR-γ agonist rosiglitazone treated cultures induced FABP4 and appeared earlier than in control cultures [33]. Overall, GEO Profiles and literatures demonstrated that FABP4 is an important adipose specific marker.

Adipose Triglyceride Lipase (ATGL)

Currently, there are 5484 ATGL GEO Profiles relative to microarray analyses in PubMed [10]. As shown in the GEO Profiles obtained from PubMed [10], GDS2818 showed that ATGL was more highly expressed in adipocytes than in preadipocytes [26]. This finding was confirmed by previous studies. Previous studies reported that porcine ATGL was more highly expressed in adipocytes than in the stromal vascular fraction which was rich in preadipocytes. In addition, ATGL was predominantly expressed in chicken fractionated adipose cells as compared to the stromal vascular fraction [34-36]. GDS2366 showed that ATGL gene expression was induced by adipocyte differentiation [37]. Kim et al.[21] stated that ATGL gene expression was dramatically increased during 3T3-L1 adipocyte differentiation. The ATGL gene was highly induced during porcine adipocyte differentiation [34]. GDS3688 illustrated that obese children had lower ATGL expression in adipose tissue [29]. Steinberg et al.[38] demonstrated that obese subjects had significantly reduced ATGL mRNA expression (P<0.05). Jocken et al.[39] reported that insulin resistant subjects have decreased ATGL mRNA expression compared to insulin sensitive subjects (P<0.05). GDS1298 showed that over expression of peroxisome proliferator-activated receptor (PPAR) gamma 2 induced ATGL gene expression during the differentiation of NIH-3T3 embryonic fibroblasts into adipocytes [40]. Kershaw et al.[41] reported that PPAR-γ played a significant role in regulation of ATGL mRNA expression in adipocytes under both *in vivo* and *in vitro* conditions. The GEO Profiles and literatures draw attention to ATGL adipose specificity and developmental changes in ATGL gene expression in adipose tissue.

A novel adipose specific candidate gene Chrdl1 (Chordin-like 1)

Now, there are 4366 Chrdl1 GEO Profiles related to microarray analyses in PubMed [10]. However, there are very limited numbers Chrdl1 GEO Profiles as to adipose tissue [10]. GDS2813 showed that Chrdl1 was highly expressed in white adipose tissue than brown adipose tissue [42]. GDS3102 stated that caloric restriction decreased Chrdl1 gene expression [43]. GDS2366 mentioned that Chrdl1 was specifically highly expressed in differentiated preadipocytes than undifferentiated preadipocytes [37]. GDS2319 indicated that high weight gainer individuals had much more Chrdl1 gene expression than low weight gainer [44].

Taken together, these GEO Profiles give informations about Chrdl1 and adipose tissue. In the current study, we gathered these informations from GEO Profiles and microarray database and revealed that the expression of Chrdl1 is highly specific both adipose tissue and adipocyte (P<0.0001). These findings are also supported with laboratory experiments. However, there is no previous study confirm these microarray data and GEO Profiles. Therefore, this paper is the first assessment of these microarray data and GEO Profiles to confirm Chrdl1 adipose specific gene expression.

In conclusion, this study aimed to put forward the potential of microarray and microarray related GEO Profiles to identify new adipose specific genes. Therefore. the depth research and detailed analysis of microarray database were performed to identify adipose specific genes. Here, we successfully tested how to effectively use microarray and GEO Profiles information to predict new tissue specific genes before conducting laboratory experiments. We have proof of concept to discover a new adipose specific gene is called Chrdl1. The primary positive impact of our study will be the future identification of a new set of adipose specific candidate genes that will provide a new platform for functional studies of these genes to enhance human health and improve livestock production efficiency. Moreover, this study also suggests that various tissue specific genes can be easily identified by detailed analysis of microarray and microarray related GEO Profiles.

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