How frequent is altered gene expression among susceptibility genes to human complex disorders?

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It is regularly thought that human complex disorder susceptibility genes show differences in gene expression between normal and pathologic tissues. Thus, differences of transcript amounts could be indicative of complex disorder susceptibility loci and, therefore, be used for the discovery or the validation of human susceptibility genes to complex disorders/traits. Whether human complex disorder susceptibility genes effectively display differences in transcript amounts was tested by meta-analysis of the published literature comparing transcript amounts of well-validated human susceptibility genes to complex traits/disorders. A total of 94 gene-disease associations, which were studied in at least three independent studies and showed strong evidence of positive association, were analyzed. For 23 out of these 94 well-validated gene-disease associations, 120 gene expression studies comparing normal and pathologic human tissues were found. For 60 out of these 120 gene expression studies, the difference of level expression between normal and pathologic human tissues was statistically significant. This result was highly significant, as only 6 significant results were expected randomly under the null hypothesis ($P < 10^{-112}$). A large excess of replication studies were also found, which were in agreement with the original report ($P = 6\times10^{-4}$). However, the overall level of expression change between normal and pathologic human tissues was relatively moderate, because only 36 (60%) and 19 (31.6%) out of the 62 statistically significant gene expression studies reached 2- or 3-fold changes in expression level, respectively. The present meta-analysis confirms statistical differences of expression levels between normal and pathologic human tissues for human susceptibility genes to complex traits/disorders. However, the levels of differences in transcript amounts appear to be relatively weak. These findings rationalize the use of gene expression for the discovery/validation of human susceptibility genes, but the weak differences of expression typically found should be taken into account for the design of such studies. ***Genet Med* 2005:7(2):83–96.**

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A number of variations of human DNA are likely to alter gene transcription rates. For instance, promoter and splice junction polymorphisms, which can change transcription rates[1,2] and RNA stability,[3] occur on average every 5.3 and 6.5 bases per kilobase of human DNA, respectively.[4] Likewise, such DNA variations can be associated with complex traits/disorders either directly or indirectly through linkage disequilibrium with susceptibility loci. Therefore, differences of transcript abundance between series of cases and controls can be indicative of association to susceptibility loci, rationalizing the use of differences of gene expression level as a surrogate marker for complex traits or disorders.[5]

The aim of the present meta-analysis was to evaluate whether complex disorder susceptibility loci show differences in gene expression between normal and affected tissues. This question was addressed by meta-analysis of all studies related to well-validated human gene-disease associations that have compared transcript amounts between series of normal and pathologic tissues. The present findings clearly demonstrate that statistical differences in transcript levels of disease susceptibility genes are found between normal and pathologic human tissues. These results rationalize the use of comparative gene expression analysis for gene discovery studies. However, the relatively weak levels of differences in transcript amounts were found, which should be taken into account for the design of gene discovery studies based on gene expression studies.

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METHODS

Literature search and inclusion criteria

To search for eligible studies, the MEDLINE citations up to May 2003 using the National Library of Medicine's PubMed online search engine were surveyed. The search was limited to English language literature. For gene-disease association retrieval, I combined the official symbol of the gene as defined by Human Gene Nomenclature or its alternative aliases, plus the

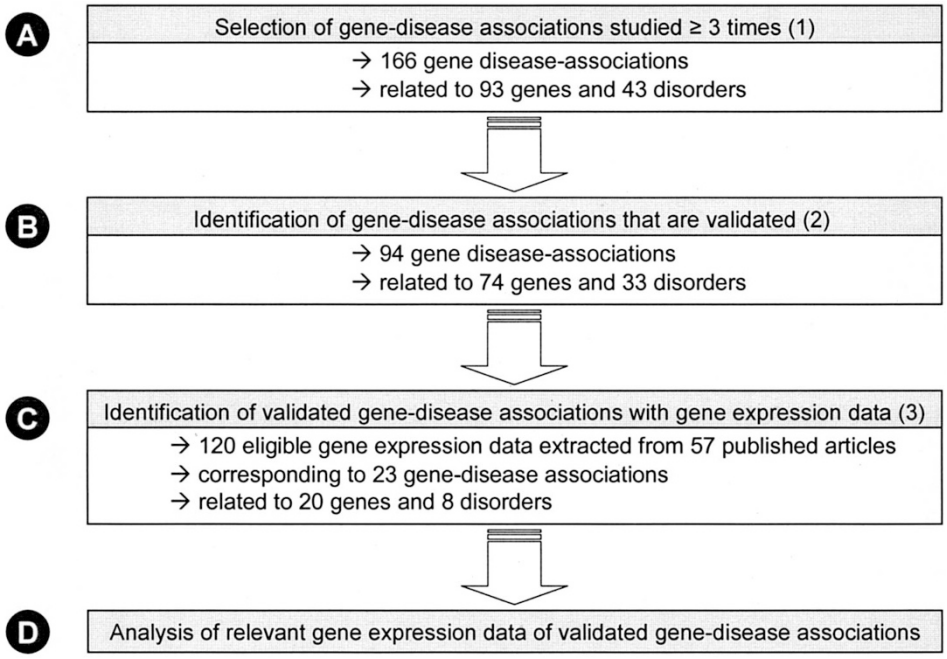

Fig. 1. Flowchart of the data analysis used for this article. A, Analysis focused on the 166 gene-disease associations reported by Hischhorn et al.[8] as being tested at least three times in independent studies. These 166 gene-disease associations were related to 93 genes and 43 disorders. B, Then, I searched for gene-disease associations fulfilling the criteria reported by Lohmueller et al.[9] as strongly predictive of replication in further allelic case-control association studies. 94 out of the 166 gene-disease associations involving 74 genes and 33 disorders fulfilled these criteria. C, Then, I searched for gene expression studies among these well-validated gene-disease associations. A total of 120 expression studies extracted from 57 articles were found. These expression studies involved 23 gene-disease associations, which were related to 20 genes and 8 disorders; (D) these 120 gene expression studies provided the material used for the present statistical meta-analysis.

name(s) of the disease, plus at least one of the keywords association or polymorphism or allele. For the selection of the gene expression studies, I combined the official symbol of the gene or its alternative aliases, plus the name of the disease, plus one of the keywords: transcript or mRNA or RNA or expression.

The abstracts from the literature search were screened to select relevant studies. The articles were read, and all relevant references cited in these studies were recovered to identify additional works unidentified in the PubMed screen.

Selection of validated gene-disease associations

The analysis is based on the 166 gene-disease associations reported by Hischhorn et al.[8] as being tested at least three times in independent studies. These 166 gene-disease associations were related to 93 genes and 43 disorders (Fig. 1). This analysis included all published associations between human common disease or dichotomous trait and common polymorphisms (defined as having a minor allele frequency over 0.01) located within or in the vicinity of the investigated genes. This review excluded the polymorphisms in HLA or blood groups, as well as association studies for substance abuse and laboratory findings. In addition, only the associations between variation at single locus and susceptibility to disease in the entire population were taken into account by Hischhorn et al.[8]

I next focused the analysis on gene-disease associations that have been well-validated in the published literature. Therefore, I have searched among the 166 previous gene-disease associations those that fulfilled the criteria reported by Lohmueller et al.[9] as strongly predictive of positive association on a sample of 25 gene-disease associations. These criteria were established on the fact that most of the 8 gene-disease associations supported by previously published meta-analysis met those criteria, whereas most of the remaining 17 did not.[9] These criteria are, in addition to the initial positive study achieving $P < 0.05$, at least two additional independent studies with $P < 0.01$ or a single study reaching $P < 0.001$. When more than one polymorphism in a gene had been studied, all polymorphisms corresponding to this gene were considered simultaneously. As a result, 94 gene-disease associations involving 74 genes and 33 disorders/traits fulfilled these conservative criteria (Fig. 1 and Table 1). Consequently, some of these genes were associated with two or more diseases (mean, 1.34; range, 1–5), such as *TNF*, which is involved in Alzheimer disease, obesity, type I diabetes, type II diabetes, and asthma. Interestingly, 33 out of these 74 genes (44.6%) were also found associated to Mendelian-inherited diseases according to the Genatlas database. This strongly suggests that some genes can be responsible at the same time of a rare Mendelian-inherited form of a disorder caused by highly penetrant alleles, but also in their much more common multigenic form through less penetrant alleles. Although the molecular mechanisms associated with these differences of inheritance have not been investigated in detail, the changes due to Mendelian-inherited disorders can reasonably be expected to have more drastic effect on gene function than the variations predisposing to common multigenic forms of the disorder.

Table 1
Gene-disease associations

Gene	Disease/trait	# OMIM	Aliases	First positive article	Mendelian disorder
A2M	Alzheimer's disease	103950	Alpha-2-macroglobulin	10	103950, emphysema with lung destruction II (macroglobulin, alpha-2-deficiency)
ABCC8	Diabetes type 2	600509	Sulfonylurea receptor; SUR	11	256450, pancreatic nesidioblastosis
ACE[a]	CAD/MI	106180	Angiotensin converting enzyme; DCP1; dipeptidyl carboxypeptidase 1	12	No
ACE	Hypertension	106180	Angiotensin converting enzyme; DCP1; dipeptidyl carboxypeptidase 1	13	No
ACE	Ischemic stroke/carotid atheroma	106180	Angiotensin converting enzyme; DCP1; dipeptidyl carboxypeptidase 1	14	No
ADD1	Hypertension	102680	Alpha adducin 1	15	No
ADRB2	Obesity	109690	Beta 2 adrenergic receptor	16	No
ADRB3	Obesity	109691	Beta 3 adrenergic receptor	17	No
AGTR1	Hypertension	106165	Angiotensin II receptor, type 1	18	No
APOB	CAD/MI	107730	Apolipoprotein B	19	200100, abetalipoproteinemia
APOC1[a]	Alzheimer's disease	107710	Apolipoprotein-C1; Apolipoprotein C1	20	No
APOE[a]	Alzheimer's disease	107741	Apolipoprotein E	21	107741, familial dysbetalipoproteinemia, hyperlipoproteinemia type III
APOE	CAD/MI	107741	Apolipoprotein E	22	107741, familial dysbetalipoproteinemia, hyperlipoproteinemia type III
APOE	Ischemic stroke	107741	Apolipoprotein E	23	107741, familial dysbetalipoproteinemia, hyperlipoproteinemia type III
APOE	Tacrine Response	107741	Apolipoprotein E	24	107741, familial dysbetalipoproteinemia, hyperlipoproteinemia type III
APOE	Overall mortality	107741	Apolipoprotein E	25	107741, familial dysbetalipoproteinemia, hyperlipoproteinemia type III
AR	Male infertility	313700	Androgen receptor	26	300068, androgen insensity syndrome; 313200, Kennedy syndrome
AR	Prostate cancer	313700	Androgen receptor	27	300068, androgen insensity syndrome; 313200, Kennedy syndrome
BCHE	Alzheimer's disease	177400	Butyrylcholinesterase	28	177400, postanesthetic apnea
CCR2	HIV infection/AIDS	610267	Chemokine (C-C motif) receptor 2; CKR2; CMKBR2	29	No
CCR5	HIV infection/AIDS	601373	Chemokine (C-C motif) receptor 5; CKR5; CMKBR5	29	No
COL1A1	Osteoporosis/fracture	120140	Collagen, type I, alpha 1	30	130060 Ehlers-Danlos syndrome TYPE VII; 166210, osteogenesis imperfecta type II; 259420 type III; and 166220 type IV
COMT[a]	Bipolar disorder	116790	Catechol-O-methyltransferase	31	No
COMT	Schizophrenia	116790	Catechol-O-methyltransferase	32	No
CTLA4	Diabetes type 1	123890	Cytotoxic T-lymphocyte-associated protein 4; CD153	33	No
CTLA4	Graves' disease	123890	Cytotoxic T-lymphocyte-associated protein 4; CD152	33	No
CTLA4	Systemic lupus erythematous	123890	Cytotoxic T-lymphocyte-associated protein 4; CD154	34	No
CYBA	CAD/MI	233690	Cytochrome b-245 alpha; p22-PHOX	35	233690, chronic granulomatous disease
CYP11B2	Hypertension	124080	Aldosterone synthase; steroid 11-beta-hydroxylase	36	203400, congenital hypoaldosteronism
CYP1A1	Breast cancer	108330	Cytochrome P450, subfamily 1A, polypeptide 3	37	No
CYP2D6	Parkinson's disease	124030	Cytochrome P450, subfamily IID, polypeptide 6	38	124030, debrisoquine sensitivity including neuroleptic malignant syndrome
CYP2E	Lung cancer	124040	Cytochrome P450, subfamily IIE; CYP2E2	39	No
DRD2[a]	Schizophrenia	126450	Dopamine receptor D2	40	159900, hereditary essential myoclonus and myoclonic dystonia
DRD3	Clozapine response	126451	Dopamine receptor D3	41	No
DRD3[a]	Schizophrenia	126451	Dopamine receptor D3	42	No
DRD4	ADHD	126452	Dopamine receptor D4	43	No
ESR1	Osteoporosis/fracture	133430	Estrogen Receptor 1	44	133430, estrogen resistance with defect of bone maturation and mineralization
F2	Deep Venous Thrombosis	176930	Prothrombin; coagulation factor II	45	176930, autosomal dominant dysprothrombinemia
F2	Ischemic stroke	176930	Prothrombin; coagulation factor II	46	176930, autosomal dominant dysprothrombinemia
F5	Deep Venous Thrombosis	227400	Coagulation factor V	47	227400, parahemophilia
F5	Pre-eclampsia	227400	Coagulation factor V	48	227400, parahemophilia
F7	CAD/MI	227500	Coagulation factor VII	49	227500, hypoproconvertinemia

Table 1
(Continued)

Gene	Disease/trait	# OMIM	Aliases	First positive article	Mendelian disorder
FCGR2A	Systemic lupus erythematous	1467901	CD32; Fc IgG low affinity IIa receptor	50	No
GCGR	Diabetes type 2	138033	Glucagon receptor	51	No
GCK	Diabetes type 2	138079	Glucokinase; MODY2; hexokinase 4	52	125853, MODY2; 602485, autosomal dominant hyperinsulinism; 601410, permanent neonatal diabetes mellitus
GNB3	Hypertension	139130	G-protein beta, polypeptide 3	53	No
GP1BA	CAD/MI	231200	Platelet glycoprotein Th, alpha polypeptide	54	231200, Bernard-Soulier syndrome
GSTM1	Bladder cancer	138350	Glutathione S-transferase MI; glutathione S-transferase mu-1	55	No
GSTM1	Lung cancer	138350	Glutathione S-transferase MI; glutathione S-transferase mu-1	56	No
GYS1[a]	Diabetes type 2	138570	Glycogen synthase (muscle); GYS	57	No
HTR2A	Anorexia	182135	5-hydroxytryptamine (serotonin) receptor 2A; HTR2	58	No
HTR2A	Clozapine response	182135	5-hydroxytryptamine receptor 2A; serotonin receptor 2A; HTR2	59	No
HTR2A[a]	Schizophrenia	182135	5-hydroxytryptamine receptor 2A; serotonin receptor 2A; HTR2	60	No
IL1RA	Osteoporosis/fracture	147810	Interleukin-1 receptor antagonist	61	No
IL4[a]	Asthma	147780	Interleukin 4	62	No
IL4R[a]	Asthma	147781	Interleukin 4 receptor	63	No
IL6	Osteoporosis/fracture	147620	Interleukin-6; IL-6	64	No
INS	Diabetes type 1	176730	Insulin	65	176730, diabetes with hyperproinsulinemia
INS	Diabetes type 2	176730	Insulin	66	176730, diabetes with hyperproinsulinemia
IRS1[a]	Diabetes type 2	147545	Insulin receptor substrate 1	67	No
ITGB3	CAD/MI	173470	Glycoprotein IIIa; integrin, beta-3; CD61	68	273800, Glanzmann thrombasthenia
KCNN3	Schizophrenia	602983	hKCa3; SKCA3; SK3; hSK3	69	No
LEP[a]	Obesity	164160	Leptin; OB	70	164160, severe early-onset obesity
LPL	CAD/MI	238600	Lipoprotein lipase	71	238600, hyperchylomicronemia
LTA	Multiple sclerosis	153440	Tumor necrosis factor beta; TNFB; Lymphotoxin-alpha	72	No
MBL2	Systemic lupus erythematous	154545	Mannose binding lectin; mannose binding protein; MBP1	73	154545, recurrent infections
MBP	Multiple sclerosis	159430	Myelin basic protein	74	No
MTHFR	CAD/MI	236250	5, 10-methylene tetrahydrofolate reductase	75	236250, developmental delay with gait
MTHFR	Deep Venous Thrombosis	236250	5,10-methylene tetrahydrofolate reductase	76	236250, developmental delay with gait
NOS3	CAD/MI	163729	Endothelial nitric oxide synthase; ENOS	77	No
PON1	CAD/MI	168820	Paraoxonase 1	78	No
PPARG[a]	Diabetes type 2	601487	Peroxisome proliferator-activated receptor gamma; PPARgamma	79	604367, insulin resistance with non insulin dependent diabetes mellitus
PPARG[a]	Obesity	601487	Peroxisome proliferator-activated receptor gamma; PPARgamma	79	604367, insulin resistance with non insulin dependent diabetes mellitus
PRNP	Creutzfeldt-Jakob disease	176640	Prion protein; PRP	80	123400, Creutzfeldt-Jakob disease
PSEN1[a]	Alzheimer's disease	104311	Presenilin 1; PS1; AD3	81	104300, Alzheimer disease
RSTN	Diabetes type 2	605565	Resistin; FIZZ3	82	No
SERPINA1	COPD/emphysema	107400	Alpha-1-antitrypsin; protease inhibitor I; PI	83	107400, severe emphysema; 107400, hemorrhagic diathesis
SERPINA3	Alzheimer's disease	107280	Alpha-1-antichymotrypsin; AACT	84	107280, severe emphysema
SERPINA8[a]	CAD/MI	106150	Angiotensinogen; AGT	85	No
SERPINA8[a]	Hypertension	106150	Angiotensinogen; AGT	86	No
SERPINE1	CAD/MI	173360	Plasminogen activator inhibitor 1; PAI 1	87	173360, bleeding diathesis; 173360, recurrent thrombosis
SLC2A1	Diabetes type 2	138140	GLUT1; glucose transporter 1	88	138140, infantile seizures with microcephaly and hypoglycorrhachia
SLC6A4[a]	Bipolar disorder	182138	SERT; Serotonin transporter; 5HTT	89	No
TNF	Asthma	191160	Tumor necrosis factor alpha; TNFA	90	No
TNF	Diabetes type 1	191160	Tumor necrosis factor alpha; TNFA	93	No
TNF[a]	Diabetes type 2	191160	Tumor necrosis factor alpha; TNFA	94	No
TNF[a]	Obesity	191160	Tumor necrosis factor alpha; TNFA	91	No

Table 1
(Continued)

Gene	Disease/trait	# OMIM	Aliases	First positive article	Mendelian disorder
TNF	Psoriasis/psoriatic arthritis	191160	Tumor necrosis factor alpha; TNFA	92	No
TP53	Head and neck cancer	191170	Tumor necrosis factor alpha; TNFA	95	151623, Li-Fraumeni syndrome
UCP1[a]	Obesity	113730	Uncoupling protein 1	96	No
UCP2[a]	Obesity	601693	Uncoupling protein 2	97	No
UCP3[a]	Obesity	602044	Uncoupling protein 3	98	602044, severe obesity and type 2 diabetes mellitus
VDR	Osteoporosis/fracture	601769	Vitamin D receptor	99	277440, vitamin D-dependent rickets
VLDLR	Alzheimer's disease	192977	Very low density lipoprotein receptor	100	No

Gene-disease associations that were studied in at least three independent studies and showed strong evidence of positive association according to the criteria reported by Lohmueller et al.[9] These criteria are, in addition to the initial positive study achieving $P < 0.05$, at least two additional independent studies with $P < 0.01$ or a single study reaching $P < 0.001$.
[a]The 23 gene-disease associations for which gene expression has been studied (see Table 2).

Gene expression studies

Subsequently, I searched for published gene expression studies among the 94 well-validated gene-disease associations (Fig. 1). All studies based on quantitative gene expression methods were used for analysis, including semiquantitative RT-PCR, competitive RT-PCR, real-time quantitative PCR, RT-PCR ELISA, branched DNA, cDNA or oligonucleotide microarrays, quantitative Northern-blot, dot-blot, RNase protection assay, serial analysis of gene expression (SAGE), and quantitative in situ hybridization. No relevant microarray data were found and used in the present analysis. In some articles, several genes and/or methods of quantification have been assayed simultaneously, the results of which were treated separately. Only the studies providing enough details to recalculate the distribution of expression in case and control groups were selected for analysis. In addition, the studies with less than five controls or five cases were excluded for analysis because poor reliability ($n = 7$). In total, 120 expression studies extracted from 57 published articles were reviewed (Fig. 1). Consequently, some articles investigated simultaneously the expression of several genes. These 120 gene expression studies were related to 23 gene-disease associations, which correspond to 20 genes and 8 disorders (Table 2). Twelve out of these 23 gene-disease associations were screened for allelic association before gene expression studies, whereas 11 were screened for allelic association following gene expression studies. In 62 out of these 120 expression studies, the values of control and case groups were established using the figures of the original publication as details were lacking in the text of the article. In these cases, each value was assessed blindly at least three times using maximum magnified copies of the figures.

Statistical analysis

For each gene expression study, the statistical difference between case and control groups was assessed by unpaired two-tailed Student's *t*-test using untransformed original variables. Statistical comparisons were considered significant at $P < 0.05$.

To test the effects of various parameters on the gene expression ratio, a univariate analysis by the linear model procedure using type III sums of squares model was performed. Multivariate analysis was achieved by logistic regression model with a forward stepwise search. In both univariate and multivariate analysis, the expression criteria used for analysis was the gene expression threshold below or above 2-fold change between case and control samples.

Statistical analyses were performed with the SPSS 11.5 software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The present meta-analysis aimed to determine whether well-validated human complex disorder susceptibility genes show differences in gene expression between normal and pathologic tissues. Therefore, the published literature was first screened to select well-validated gene-disease associations for complex human traits/disorders. The analysis was based on a set of 166 gene-disease associations reported by Hischhorn et al.[8] as being studied in at least three different studies. Then, the published literature up to May 2003 was screened to select which of these 166 gene-associations fulfilled the criteria reported by Lohmueller et al.[9] as strongly predictive of future replication. A total of 74 genes and 33 disorders involved in 94 gene-disease associations fulfilled these criteria (Fig. 1 and Table 1).

Then, these genetically well-validated genes were analyzed for differential transcript expression in pathologic conditions. In total, 120 expression records involved in 23 gene-disease associations were found (Fig. 1). For each study, the statistical difference of gene expression was calculated by unpaired two-tailed Student *t* test using the original data set (Table 2). From these 120 gene expression studies, 60 (50%) achieved $P < 0.05$, which should be compared to 6.0 significant results expected randomly under the null hypothesis of no gene expression change. This result was highly significant, as the nominal value obtained by reference to the χ^2-distribution was 513.4 (χ^2-test, 1 degree of freedom; $P < 10^{-112}$). In theory, publication bias

Table 2
Details of gene expression studies comparing normal and pathological human tissues for the 23 well-validated human susceptibility genes to complex disorders/traits (see Table 1)

Gene	Disease/trait	Country	mRNA isoform	Tissue	Main phenotype of controls	No. of controls	Main phenotype of cases	No. of cases	Method of measure	Ratio	*P* value	Ref.
ACE	CAD/MI	Italy	Canonical	Heart, left ventricular wall	Controls with normal coronarography	21	Individuals with stable angina	32	In-situ hybridization	1,26	5.61E-02	101
ACE	CAD/MI	Italy	Canonical	Heart, left ventricular wall	Controls with normal coronarography	21	Individuals with unstable angina	35	In-situ hybridization	2,02	1.16E-05	101
APOC1	Alzheimer's disease	Canada	Canonical	Brain, prefrontal cortex	Controls without dementia and neuropathological change to Alzheimer disease	11	Individuals with Alzheimer disease with neuropathological changes	19	RNase Protection Assay	0,57	5.55E-02	102
APOE	Alzheimer's disease	Japan	Canonical	Skin fibroblasts	Controls without dementia	10	Individuals with Alzheimer disease according to DSM-IIIR criteria	18	Quantitative Northern-blot	0,63	1.04E-03	103
APOE	Alzheimer's disease	Canada	Canonical	Brain, prefrontal cortex	Controls without dementia and neuropathological change to Alzheimer disease	11	Individuals with Alzheimer disease with neuropathological changes	20	RNase Protection Assay	0,65	1.78E-02	102
APOE	Alzheimer's disease	Japan	Canonical	Brain, temporal cortex	Controls with neurological disease without dementia	20	Individuals with sporadic Alzheimer disease	27	Quantitative RT-PCR	1,42	3,24E-02	104
APOE	Alzheimer's disease	USA	Canonical	Brain, hippocampus	Controls with non-Alzheimer neurological disorder	9	Individuals with Alzheimer disease	7	In-situ hybridization	1,70	2.51E-09	105
COMT	Schizophrenia	USA	Canonical	Brain, frontal superior gyrus	Controls without history of psychiatric disorder	10	Schizophrenia not treated with antipsychotics	14	Competitive RT-PCR	2,13	8.85E-02	106
COMT	Schizophrenia	USA	Canonical	Brain, middle temporal gyrus	Controls without history of psychiatric disorder	10	Schizophrenia not treated with antipsychotics	13	Competitive RT-PCR	0,50	4.72E-02	106
COMT	Schizophrenia	USA	Canonical	Brain, superior temporal gyrus	Controls without history of psychiatric disorder	10	Schizophrenia not treated with antipsychotics	9	Competitive RT-PCR	0,74	4.87E-01	106
DRD2	Schizophrenia	United Kingdom	DRD2 short isoform	Brain, ventro orbital gyrus	Controls with no details	11	Schizophrenics with no details	11	Quantitative RT-PCR	1,39	8.72E-03	107
DRD2	Schizophrenia	United Kingdom	DRD2 long isoform	Brain, ventro orbital gyrus	Controls with no details	11	Schizophrenics with no details	11	Quantitative RT-PCR	1,55	2.84E-03	107
DRD2	Schizophrenia	United Kingdom	DRD2 short isoform	Brain, inferior temporal gyrus	Controls with no details	13	Schizophrenics with no details	9	Quantitative RT-PCR	1,39	2.10E-02	107
DRD2	Schizophrenia	United Kingdom	DRD2 long isoform	Brain, inferior temporal gyrus	Controls with no details	13	Schizophrenics with no details	9	Quantitative RT-PCR	1,38	5.38E-02	107
DRD2	Schizophrenia	United Kingdom	DRD2 short isoform	Brain, polar frontal cortex	Controls with no details	19	Schizophrenics with no details	13	Quantitative RT-PCR	0,97	7.75E-01	107
DRD2	Schizophrenia	United Kingdom	DRD2 long isoform	Brain, polar frontal cortex	Controls with no details	19	Schizophrenics with no details	13	Quantitative RT-PCR	0,97	8.08E-01	107
DRD2	Schizophrenia	United Kingdom	DRD2 short isoform	Brain, caudate nucleus	Controls with no details	15	Schizophrenics with no details	9	Quantitative RT-PCR	1,60	6.79E-02	107
DRD2	Schizophrenia	United Kingdom	DRD2 long isoform	Brain, caudate nucleus	Controls with no details	15	Schizophrenics with no details	9	Quantitative RT-PCR	1,98	7.03E-02	107
DRD2	Schizophrenia	Canada	DRD2 long and short isoforms	Brain, frontal cortex	Controls without psychiatric disorder	7	Schizophrenics according to DSM-IV criteria treated or not with neuroleptics	11	Competitive RT-PCR	0,20	7.44E-03	108
DRD2	Schizophrenia	Canada	DRD2 long isoform	Brain, frontal cortex	Controls without psychiatric disorder	7	Schizophrenics according to DSM-IV criteria treated or not with neuroleptics	7	Competitive RT-PCR	1,86	6.05E-02	108
DRD3	Schizophrenia	Israel	Canonical	Lymphocytes	Controls without psychiatric disorder according to DSM-IV criteria	6	Schizophrenics according to DSM-IV criteria treated or not	6	PCR-Elisa	0,81	3.01E-01	109
DRD3	Schizophrenia	Israel	Canonical	Lymphocytes	Controls without psychiatric disorder according to DSM-IV criteria	14	Schizophrenics according to DSM-IV criteria treated or not	14	Quantitative RT-PCR	3,70	5.77E-02	109
DRD3	Schizophrenia	Korea	Canonical	Lymphocytes	Controls without psychiatric disorder according to DSM-IV criteria	31	Schizophrenics naive or free of neuroleptic therapy	43	Real-time RT-PCR	7,32	1.28E-24	110
GYS1	Diabetes type 2	France	Canonical	Skeletal muscle	Controls with normal glucose tolerance and no family history of diabetes	9	Diabetics type 2	8	Competitive RT-PCR	0,56	1.90E-02	111
GYS1	Diabetes type 2	France	Canonical	Skeletal muscle	Lean controls with normal glucose tolerance	17	Diabetics type 2	9	Competitive RT-PCR	0,73	2.37E-01	112
GYS1	Diabetes type 2	France	Canonical	Skeletal muscle	Obese individuals with normal glucose tolerance	9	Diabetics type 2	9	Competitive RT-PCR	0,66	1.17E-01	112

Table 2
(Continued)

Gene	Disease/trait	Country	mRNA isoform	Tissue	Main phenotype of controls	No. of controls	Main phenotype of cases	No. of cases	Method of measure	Ratio	*P* value	Ref.
GYS1	Diabetes type 2	USA	Canonical	Skeletal muscle	Non-diabetic controls, non-obese	8	Diabetics type 2 with obesity	11	RNase protection assay	0,69	5.09E-02	113
GYS1	Diabetes type 2	Sweden	Canonical	Skeletal muscle	Non-diabetic controls	12	Diabetics type 2	12	Primer-dropping RT-PCR	1,43	9.21E-03	114
GYS1	Diabetes type 2	Finland	Canonical	Skeletal muscle	Non-diabetic controls	17	Diabetes type 2	14	Dot-blot	0,88	4.90E-02	115
GYS1	Diabetes type 2	Denmark	Canonical	Skeletal muscle	Controls with normal glucose tolerance and no family history of diabetes	14	Diabetics type 2	14	Quantitative Northern-blot	0,72	6.81E-09	116
GYS1	Diabetes type 2	Denmark	Canonical	Skeletal muscle	Controls without diabetes and family history of diabetes	19	Diabetics type 2	19	Quantitative Northern-blot	0,73	3.76E-03	117
HTR2A	Schizophrenia	United Kingdom	Canonical	Brain, prefrontal cortex	Controls with no details	12	Schizophrenics with no details	12	In-situ hybridization	0,52	1.13E-04	118
HTR2A	Schizophrenia	United Kingdom	Canonical	Brain, cingulate cortex	Controls with no details	11	Schizophrenics with no details	9	In-situ hybridization	0,37	6.42E-04	118
HTR2A	Schizophrenia	United Kingdom	Canonical	Brain, dentate gyrus	Controls with no details	13	Schizophrenics with no details	11	In-situ hybridization	0,39	1.05E-03	118
HTR2A	Schizophrenia	United Kingdom	Canonical	Brain, superior temporal gyrus	Controls with no details	13	Schizophrenics with no details	7	In-situ hybridization	0,52	9.57E-03	118
HTR2A	Schizophrenia	United Kingdom	Canonical	Brain, parahippocampal gyrus	Controls with no details	8	Schizophrenics with no details	10	In-situ hybridization	0,86	4.14E-01	118
HTR2A	Schizophrenia	USA	Canonical	Brain, superior frontal gyrus	Controls without psychiatric disorder according to DSM-IIIR criteria	14	Schizophrenics according to DSM-IIIR criteria untreated with neuroleptics (> 26 weeks)	7	Competitive RT-PCR	0,40	3.31E-02	119
IL4	Asthma	USA	IL4 delta2 isoform	Bronchial samples	Non-asthmatic controls with or without atopy	7	Asthmatics with or without atopy	9	Quantitative RT-PCR	3,49	8.23E-02	120
IL4	Asthma	USA	Canonical	Bronchial samples	Non-asthmatic controls with or without atopy	7	Asthmatics with or without atopy	9	Competitive RT-PCR	0,78	4.88E-01	120
IL4	Asthma	Switzerland	Canonical	Bronchial samples	Non-atopic controls	8	Non-atopic asthma	10	Competitive RT-PCR	2,60	6.70E-03	121
IL4	Asthma	Switzerland	Canonical	Bronchial samples	Atopic controls	9	Atopic asthmatics	10	Quantitative RT-PCR	2,08	1.61E-01	121
IL4	Asthma	Switzerland	Canonical	Bronchial samples	Non-atopic controls	6	Non-atopic asthmatics	10	Quantitative RT-PCR	11,30	4.20E-03	121
IL4	Asthma	Switzerland	Canonical	Bronchial samples	Atopic controls	5	Atopic asthmatics	9	In-situ hybridization	4,77	1.73E-01	121
IL4	Asthma	Canada	Canonical	Bronchial samples	Non-atopic controls	8	Atopic asthmatics	9	In-situ hybridization	5,92	3.96E-05	122
IL4	Asthma	Canada	Canonical	Bronchial samples, airways < 2 mm	Non-asthmatics controls, bronchial carcinoma	10	Asthmatics with bronchial carcinoma	6	In-situ hybridization	6,27	4.46E-08	123
IL4	Asthma	Canada	Canonical	Bronchial samples, airways > 2 mm	Non-asthmatics controls, bronchial carcinoma	10	Asthmatics with bronchial carcinoma	6	In-situ hybridization	5,43	1.40E-08	123
IL4	Asthma	Canada	Canonical	Sputum	Non-atopic non asthmatic controls	10	Atopic asthmatics	13	In-situ hybridization	8,85	3.77E-08	124
IL4	Asthma	United Kingdom	Canonical	Bronchoalveolar lavage cells	Non-atopic non-asthmatic controls	10	Atopic asthmatics	10	In-situ hybridization	5,75	4.02E-07	125
IL4R	Asthma	Canada	Canonical	Bronchial samples	Atopic non-asthmatic controls	7	Atopic asthmatics	8	In-situ hybridization	2,25	2.89E-04	126
IL4R	Asthma	Canada	Canonical	Bronchial samples	Non-atopic non-asthmatic controls	6	Non-atopic asthmatics	9	In-situ hybridization	2,12	6.90E-03	126
IRS1	Diabetes type 2	France	Canonical	Skeletal muscle	Controls with normal glucose tolerance and no family history of diabetes	9	Diabetics type 2	8	In-situ hybridization	0,60	4.29E-02	111
IRS1	Diabetes type 2	France	Canonical	Skeletal muscle	Lean controls with normal glucose tolerance	17	Diabetics type 4	9	Competitive RT-PCR	1,32	2.97E-01	112
IRS1	Diabetes type 2	France	Canonical	Skeletal muscle	Obese individuals with normal glucose tolerance	9	Diabetes type 2	9	Competitive RT-PCR	1,10	6.45E-01	112
LEP	Obesity	Canada	Canonical	Omental fat	Lean controls (BMI < 27 kg/m^2)	11	Obese individuals (BMI > 30 kg/m^2)	12	Competitive RT-PCR	2,68	1.32E-08	127
LEP	Obesity	Austia	Canonical	Omental fat	Lean controls without major illness	17	Obese individuals (BMI > 30 kg/m^2)	111	Quantitative RT-PCR	2,78	1.23E-03	128
LEP	Obesity	United Kingdom	Canonical	Omental fat	Lean controls (BMI < 25 kg/m^2) without diabetes	14	Obese or overweight individuals (BMI > 27 kg/m^2) without diabetes	9	Quantitative Northern-blot	1,80	8.18E-02	129
LEP	Obesity	USA	Canonical	Subcutaneous fat	Lean controls (BMI < 25 kg/m^2)	8	Obese individuals (BMI > 30 kg/m^2)	8	Quantitative RT-PCR	1,72	1.64E-03	130

Table 2
(Continued)

Gene	Disease/trait	Country	mRNA isoform	Tissue	Main phenotype of controls	No. of controls	Main phenotype of cases	No. of cases	Method of measure	Ratio	*P* value	Ref.
LEP	Obesity	USA	Canonical	Subcutaneous fat	Lean controls (BMI < 27/kg/m^2 for females and < 27.3/kg/m^2 for males) without diabetes	27	Obese individuals without diabetes	27	Quantitative RT-PCR	1,54	3.81E-04	131
LEP	Obesity	Austia	Canonical	Subcutaneous fat	Lean controls without major illness	19	Obese individuals (BMI > 30 kg/m^2)	89	Quantitative RT-PCR	2,08	4.13E-04	128
LEP	Obesity	Sweden	Canonical	Subcutaneous fat	Lean women (BMI < 27 kg/m^2) with no major illness	11	Obese women (BMI > 30 kg/m^2) with no major illness	20	Quantitative Northern-blot	2,54	3.58E-05	132
LEP	Obesity	United Kingdom	Canonical	Subcutaneous fat	Lean controls (BMI < 25 kg/m^2) without diabetes	14	Obese or overweight individuals (BMI > 27/kg/m^2) without diabetes	9	In-situ hybridization	1,98	1.52E-02	129
LEP	Obesity	France	Canonical	Subcutaneous fat	Lean controls (BMI < 25 kg/m^2)	12	Obese individuals (BMI 30 > kg/m^2)	7	Quantitative RT-PCR	7,70	2.63E-07	133
LEP	Obesity	China	Canonical	Subcutaneous fat	Lean controls (BMI < 24 kg/m^2)	11	Obese or overweight individuals (BMI > 27 kg/m^2) without diabetes	12	Quantitative RT-PCR	1,78	2.78E-03	134
PPARG	Diabetes type 2	France	PPARG isoform 1	Subcutaneous fat	Lean non-diabetic individuals	10	Diabetics type 2	9	Dot-blot	1,32	2.12E-01	135
PPARG	Diabetes type 2	France	PPARG isoform 1	Subcutaneous fat	Obese non-diabetic individuals	10	Diabetics type 2	9	Competitive RT-PCR	1,12	5.10E-01	135
PPARG	Diabetes type 2	France	PPARG isoform 2	Subcutaneous fat	Lean non-diabetic individuals	10	Diabetics type 2	9	Competitive RT-PCR	0,69	2.80E-02	135
PPARG	Diabetes type 2	France	PPARG isoform 2	Subcutaneous fat	Obese non-diabetic individuals	10	Diabetics type 2	9	Competitive RT-PCR	0,92	6.03E-01	135
PPARG	Diabetes type 2	USA	Canonical	Skeletal muscle	Lean controls (BMI < 27 kg/m^2) without diabetes	7	Diabetics type 2	7	Competitive RT-PCR	1,41	1.89E-01	136
PPARG	Diabetes type 2	USA	Canonical	Skeletal muscle	Obese individuals without diabetes	6	Diabetics type 2	7	RNase protection assay	1,12	7.45E-01	136
PPARG	Diabetes type 2	USA	Canonical	Skeletal muscle	Lean controls (BMI < 27 kg/m^2) with no diabetes	6	Diabetics type 2	5	RNase protection assay	2,98	4.20E-05	137
PPARG	Diabetes type 2	USA	Canonical	Skeletal muscle	Obese individuals (BMI > 30 kg/m^2) with normal glucose tolerance	5	Diabetics type 2	5	Competitive RT-PCR	1,20	1.84E-01	137
PPARG	Obesity	Austia	Canonical	Omental fat	Lean controls without major illness	20	Obese individuals (BMI > 30 kg/m^2)	76	Competitive RT-PCR	0,97	7.98E-01	128
PPARG	Obesity	USA	Canonical	Skeletal muscle	Lean controls (BMI < 27 kg/m^2) without diabetes	7	Obese individuals without diabetes	6	Quantitative Northern-blot	1,27	5.12E-01	136
PPARG	Obesity	USA	Canonical	Skeletal muscle	Lean controls (BMI < 27 kg/m^2) with no diabetes	6	Obese inviduals (BMI >30 kg/m^2) with normal glucose tolerance	5	RNase protection assay	2,49	6.04E-05	137
PPARG	Obesity	France	PPARG isoform 1	Subcutaneous fat	Lean non-diabetic individuals	10	Obese non-diabetic individuals	10	Competitive RT-PCR	1,18	4.31E-01	135
PPARG	Obesity	France	PPARG isoform 2	Subcutaneous fat	Lean non-diabetic individuals	10	Obese non-diabetic individuals	10	Competitive RT-PCR	0,75	1.13E-01	135
PPARG	Obesity	France	PPARG isoform 1	Subcutaneous fat	Lean non-diabetic individuals	5	Obese non-diabetic individuals	5	Competitive RT-PCR	1,37	2.95E-01	135
PPARG	Obesity	France	PPARG isoform 2	Subcutaneous fat	Lean non-diabetic individuals	5	Obese non-diabetic individuals	5	Competitive RT-PCR	0,88	6.42E-01	135
PPARG	Obesity	Austia	Canonical	Subcutaneous fat	Lean controls without major illness	19	Obese individuals (BMI > 30 kg/m^2)	50	Competitive RT-PCR	1,01	9.32E-01	128
PPARG	Obesity	USA	PPARG isoform 1	Subcutaneous fat	Lean individuals (BMI < 25 kg/m^2) without diabetes or malignancies	11	Obese or overweight individuals (BMI > 27 kg/m^2) without diabetes or malignancies	11	Competitive RT-PCR	0,30	4.25E-03	138
PPARG	Obesity	USA	PPARG isoform 2	Subcutaneous fat	Lean individuals (BMI < 25 kg/m^2) without diabetes or malignancies	11	Obese or overweight individuals (BMI > 27 kg/m^2) without diabetes or malignancies	11	Competitive RT-PCR	1,55	4.66E-02	138
PPARG	Obesity	USA	PPARG isoform 2	Subcutaneous fat	Lean (BMI < 25 kg/m^2)	14	Morbid obesity	24	Competitive RT-PCR	0,65	2.45E-05	139
PSEN1	Alzheimer's disease	Sweden	Canonical	Brain, frontal cortex	Controls affected or not with non-Alzheimer neurological diseases	9	Individuals with Alzheimer disease with neuropathological changes	14	Competitive RT-PCR	0,99	9.18E-01	140
PSEN1	Alzheimer's disease	Japan	Canonical	Skin fibroblasts	Controls with neurological disease without dementia	10	Individuals with Alzheimer disease according to DSM-IIIR criteria	18	Quantitative Northern-blot	2,25	3.32E-02	141

Table 2
(Continued)

Gene	Disease/trait	Country	mRNA isoform	Tissue	Main phenotype of controls	No. of controls	Main phenotype of cases	No. of cases	Method of measure	Ratio	*P* value	Ref.
PSEN1	Alzheimer's disease	Japan	Canonical	Brain, temporal cortex	Controls with neurological disease with no dementia	23	Individuals with Alzheimer disease with neuropathological changes	17	Real-time RT-PCR	1,94	6.09E-05	142
PSEN1	Alzheimer's disease	Sweden	Canonical	Brain, temporal cortex	Controls affected or not with non-Alzheimer neurological diseases	8	Individuals with Alzheimer disease with neuropathological changes	14	RNase protection assay	0,88	4.42E-01	140
SERPINA 8	CAD/MI	Italy	Canonical	Heart, left ventricular wall	Controls with normal coronarography	21	Individuals with stable angina	32	RNase protection assay	0,92	6.70E-01	101
SERPINA 8	CAD/MI	Italy	Canonical	Heart, left ventricular wall	Controls with normal coronarography	21	Individuals with unstable angina	35	In-situ hybridization	1,88	9.70E-08	101
SERPINA 8	Hypertension	Australia	Canonical	Subcutaneous fat	Controls with normal tension and no family history	6	Individuals with isolated systolic hypertension	8	No data	0,12	1.20E-01	143
SLC6A4	Bipolar disorder	USA	Canonical	Brain, frontal cortex	Controls with no psychiatric disorder (no details)	15	Bipolar disorder according to DSM-IV criteria	19	No data	4,57	2,16E-10	144
TNF	Diabetes type 2	Germany	Canonical	Subcutaneous fat	Lean, no diabetes	12	Diabetics type 2 or individuals with impaired glucose tolerance	10	In-situ hybridization	2,36	3.99E-02	145
TNF	Diabetes type 2	Germany	Canonical	Subcutaneous fat	Obese individuals with normal glucose tolerance	18	Diabetic type 2 or individuals with impaired glucose tolerance	10	Quantitative Northern-blot	1,57	8.82E-02	145
TNF	Obesity	USA	Canonical	Subcutaneous fat	Lean controls with no diabetes	6	Obese individuals	9	Quantitative RT-PCR	2,60	1.11E-03	146
TNF	Obesity	Germany	Canonical	Subcutaneous fat	Lean, no diabetes	12	Obese individuals with normal glucose tolerance	18	No data	1,50	2.32E-02	145
TNF	Obesity	USA	Canonical	Subcutaneous fat	Lean controls (BMI $<$ 25/kg/m^2)	8	Obese or overweight individuals (BMI $>$ 27 kg/m^2)	31	No data	1,66	1.59E-01	147
TNF	Obesity	USA	Canonical	Subcutaneous fat	Lean controls (BMI $<$ 25 kg/m^2)	9	Obese or overweight individuals (BMI $>$ 25 kg/m^2)	41	In-situ hybridization	2,15	4.81E-05	148
TNF	Obesity	Finland	Canonical	Subcutaneous fat	Lean individuals without diabetes	20	Obese or morbid obese individuals (BMI $>$ 30 kg/m^2) without diabetes	61	No data	1,39	4.80E-02	149
UCP1	Obesity	Austia	Canonical	Omental fat	Lean controls (no details)	14	Obese individuals (no details)	78	Quantitative RT-PCR	0,50	5.83E-05	150
UCP1	Obesity	Austia	Canonical	Subcutaneous fat	Lean controls (no details)	10	Obese individuals (no details)	23	No data	1,22	5.02E-01	150
UCP2	Obesity	Austia	Canonical	Omental fat	Lean controls (BMI $<$ 27 kg/m^2)	28	Obese individuals (BMI $>$ 30 kg/m^2)	95	No data	0,67	4.92E-08	151
UCP2	Obesity	France	Canonical	Skeletal muscle	Lean controls	6	Obese individuals	5	Competitive RT-PCR	1,24	5.29E-01	152
UCP2	Obesity	France	Canonical	Skeletal muscle	Lean controls	6	Obese individuals	6	Competitive RT-PCR	0,98	9.32E-01	152
UCP2	Obesity	Sweden	Canonical	Skeletal muscle	Lean controls without diabetes	6	Obese individuals free of drug therapy	6	Competitive RT-PCR	0,72	1.04E-03	153
UCP2	Obesity	Spain	Canonical	Skeletal muscle	Lean controls without diabetes	10	Obese individuals (BMI $>$ 30 kg/m^2) with normal glucose tolerance	14	Competitive RT-PCR	0,97	8.49E-01	154
UCP2	Obesity	USA	Canonical	Skeletal muscle	Lean controls (BMI $<$ 27 kg/m^2)	14	Obese individuals (BMI $>$ 27 kg/m^2)	16	No data	0,93	5.63E-01	155
UCP2	Obesity	France	Canonical	Subcutaneous fat	Lean controls	6	Obese individuals	6	No data	1,46	2.52E-01	152
UCP2	Obesity	France	Canonical	Subcutaneous fat	Lean controls	5	Obese individuals	5	Competitive RT-PCR	1,31	2.84E-01	152
UCP2	Obesity	Austia	Canonical	Subcutaneous fat	Lean controls (BMI $<$ 27 kg/m^2)	19	Obese individuals (BMI $>$ 30 kg/m^2)	82	Competitive RT-PCR	0,92	2.76E-01	151
UCP2	Obesity	USA	Canonical	Subcutaneous fat	Lean controls (BMI $<$ 27 kg/m^2)	17	Obese individuals (BMI $>$ 27 kg/m^2)	17	Competitive RT-PCR	0,93	7.13E-01	155
UCP3	Obesity	Austia	UCP3 long isoform	Skeletal muscle	Lean controls, no diabetes	15	Morbid obesity treated by gastric banding, no diabetes	38	Competitive RT-PCR	1,32	3.39E-02	156
UCP3	Obesity	Austia	UCP3 short isoform	Skeletal muscle	Lean controls, no diabetes	15	Morbid obesity treated by gastric banding, no diabetes	38	Competitive RT-PCR	1,31	6.61E-02	156
UCP3	Obesity	France	UCP3 short isoform	Skeletal muscle	Lean controls without diabetes	9	Obese individuals without diabetes	7	In-situ hybridization	0,73	4.62E-01	157
UCP3	Obesity	France	UCP3 long isoform	Skeletal muscle	Lean controls without diabetes	9	Obese individuals without diabetes	7	Competitive RT-PCR	1,10	8.09E-01	157
UCP3	Obesity	Sweden	Canonical	Skeletal muscle	Lean controls without diabetes	6	Obese individuals free of drug therapy	6	RNase protection assay	1,02	8.21E-01	153
UCP3	Obesity	USA	UCP3 long isoform	Skeletal muscle	Lean controls (BMI $<$ 27 kg/m^2)	14	Obese individuals (BMI $>$ 27 kg/m^2)	16	Competitive RT-PCR	1,15	3.54E-01	155
UCP3	Obesity	USA	UCP3 short isoform	Skeletal muscle	Lean controls (BMI $<$ 27 kg/m^2)	14	Obese individuals (BMI $>$ 27 kg/m^2)	16	Competitive RT-PCR	1,08	5.95E-01	155
UCP3	Obesity	France	Canonical	Skeletal muscle	Lean controls	5	Obese individuals	5	Competitive RT-PCR	1,42	1.86E-01	152

Table 2
(Continued)

Gene	Disease/trait	Country	mRNA isoform	Tissue	Main phenotype of controls	No. of controls	Main phenotype of cases	No. of cases	Method of measure	Ratio	*P* value	Ref.
UCP3	Obesity	France	Canonical	Skeletal muscle	Lean controls	8	Obese individuals	6	RNase protection assay	0,74	2.29E-01	152
UCP3	Obesity	Spain	Canonical	Skeletal muscle	Lean controls without diabetes	10	Obese individuals (BMI > 30 kg/m^2) with normal glucose tolerance	14	RNase protection assay	1,06	7.75E-01	154
UCP3	Obesity	Austia	UCP3 short isoform	Skeletal muscle	Lean controls, no diabetes	15	Morbid obesity treated by gastric banding, no diabetes	38	RNase protection assay	1,31	6.61E-02	156
UCP3	Obesity	France	UCP3 short isoform	Skeletal muscle	Lean controls without diabetes	9	Obese individuals without diabetes	7	Competitive RT-PCR	0,73	4.62E-01	157
UCP3	Obesity	France	UCP3 long isoform	Skeletal muscle	Lean controls without diabetes	9	Obese individuals without diabetes	7	Competitive RT-PCR	1,10	8.09E-01	157
UCP3	Obesity	Sweden	Canonical	Skeletal muscle	Lean controls without diabetes	6	Obese individuals free of drug therapy	6	In-situ hybridization	1,02	8.21E-01	153
UCP3	Obesity	USA	UCP3 long isoform	Skeletal muscle	Lean controls (BMI < 27 kg/m^2)	14	Obese individuals (BMI > 27 kg/m^2)	16	RNase protection assay	1,15	3.54E-01	155
UCP3	Obesity	USA	UCP3 short isoform	Skeletal muscle	Lean controls (BMI < 27 kg/m^2)	14	Obese individuals (BMI > 27 kg/m^2)	16	RNase protection assay	1,08	5.95E-01	155
UCP3	Obesity	France	Canonical	Skeletal muscle	Lean controls	5	Obese individuals	5	Competitive RT-PCR	1,42	1.86E-01	152

toward positive results could explain such difference. However, the number published and unpublished nonsignificant studies required to account for these results should be at least 19-fold higher than the number of significant studies, i.e., 1140 studies (95% CI: 851–1428). Because there were only 60 nonsignificant published studies, one must postulate the existence of 1080 unpublished negative studies (95% CI: 791–1368), which appear frankly unrealistic. The ratio of statistically significant gene expression studies was similar between the genes also known to be involved in Mendelian-inherited disorders or not (27 out of 58 vs. 31 out of 62; χ^2-test, 1 degree of freedom: 0.4; $P = 0.46$). The ratio of statistically significant gene expression studies was also similar for the gene-disease associations tested by association before expression studies or after (20 out of 33 vs. 40 out of 87; χ^2-test, 1 degree of freedom: 1.3; $P = 0.15$). Furthermore, out of the 60 studies leading to statistically significant results, 15 were first reports and 45 were replications of previous studies. Thirty-four of these replications reports showed statistically significant difference in expression in agreement with the original report, whereas only 11 showed a statistically significant change in transcript levels but in the opposite direction to that described in the first report. These figures are significantly different from the 22.5 studies expected to occur randomly in the same and opposite direction (95% CI: 19.0–26.0; χ^2-test, 1 degree of freedom: 11.7; $P = 6\times10^{-4}$).

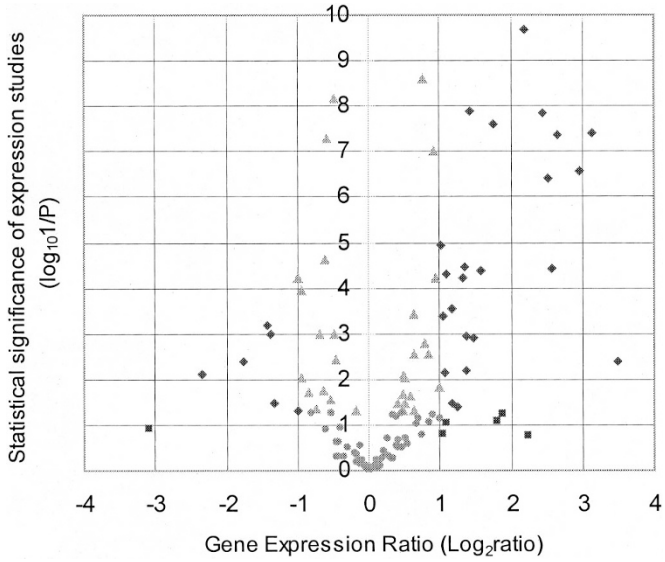

Fig. 2. Volcano-plot of expression ratio against significance of gene expression studies between case and control samples. Each symbol indicates the results of a published genetic expression study. Ratios of mRNA expression for each gene are reported as $\log_2$ of the quotient of case series to control series. Statistical significance of expression between populations is expressed as the $\log_{10}$ of the inverse *P* value from the unpaired two-tailed Student's *t*-test. Red squares represent studies with a change of expression over 2-fold ($\log_2$ ratio > 1 or $\log_2$ ratio < -1) that are not statistically significant ($\log_{10}1/P < 1.3$); brown diamonds correspond to studies with expression change over 2-fold cutoff that are statistically significant; blue triangles indicate studies with changes under 2-fold that are statistically significant; and green circles indicate studies with changes under 2-fold that are not statistically significant.

Table 3
Univariate ANOVA analysis for the effect of various parameters on gene expression change

Source	Type III Sum of Squares	df	Mean Square	F-statistics	Significance
Gene-disease association	5,42	20	0,27	2,36	0.003
Tissue analyzed	1,70	7	0,24	2,15	0.05
Method for expression analysis	1,68	8	0,21	1,83	0.08
Intercept	5,42	20	0,27	2,36	0.0003
Residual	9,52	83	0,11		
Total	35	120			
Corrected Total	24,79	119			

Several sources of bias, related to the heterogeneity of the expression studies presently analyzed, can impact the results of the present meta-analysis. For instance, some susceptibility genes have been tested by allelic association after gene expression analysis, while others were tested before. Although this was not found to impact the results of the present analysis, the frequency of gene expression changes could be overestimated in the subset of genes initially tested for gene expression. Furthermore, the present results could be altered by the fact that some results of gene expression were not independent one from the other, as several samples or genes were analyzed in the same studies. To test this hypothesis, the subset of 27 strictly independent gene expression studies was individually reanalyzed. Positive results were still obtained, as 24 out of the 27 gene expression studies were statistically significant (89%) exceeding the number of 1.35 predicted by chance ($P = 10^{-28}$, binomial distribution). In summary, although the strength of the present statistical analysis supports the validity of the present findings, they should be taken cautiously and require further confirmation.

Actually, the method based on comparing the expression level between series of cases and controls for human susceptibility genes shows a major limitation: only 36 (60.0%) and 19 (31.7%) out of the 60 statistically significant gene expression studies reached 2- or 3-fold changes in expression level, respectively (Fig. 2). In contrast, only 6 (10%) and 3 (5.0%) out of the 60 non–statistically significant studies reach these respective thresholds. Therefore, 2- or 3-fold change expression thresholds have in the present study a sensitivity of 50% and 23.5%, respectively. Consequently, the reliability of testing difference in mRNA abundance can be seriously impacted by the weak differences in transcript abundance between cases and controls.

To test the impact of various methodological parameters on gene expression change, I next performed a univariate ANOVA analysis (Table 3). The gene ($P = 0.003$) and the nature of tissue analyzed ($P = 0.05$) were associated with gene expression change between cases and controls, whereas the method of assay to measure transcript abundance had no significant effect ($P = 0.08$). Multivariate analysis by logistic regression model with a forward stepwise search was performed to assess the relative influence of the univariate factors on gene expression ratio. Both two previous factors were found to be independently associated with gene expression ratio, the gene having a strongest effect ($P = 0.002$), while the type of tissue used for expression analysis was more weakly associated ($P = 0.01$). Therefore, because of the influence of the gene itself and the tissue analyzed, the reliability of differential expression analysis is expected to vary strongly from one gene or one tissue to another.

Taken together, the present results demonstrate significant differences in transcript levels between normal and pathologic tissues of human susceptibility genes. These results rationalize the use of comparative gene expression analysis for gene discovery studies. However, differences in transcript amounts appear much lower than those typically found between inbred environmentally controlled animal models.[6,7] These weak differences should be taken into account for the design of gene susceptibility studies using differences of transcript amounts as a tool[7–49] for gene[50–99] discovery/validation.[100–157]

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