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Expression profile analysis of human peripheral blood mononuclear cells in response to aspirin

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B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Sunghee Choi[1 ABCDEF], Hae-Sim Park[2 BG], Myeong Sook Cheon[1 EF] and Kyunglim Lee[1 AG]

[1] College of Pharmacy, Center for Cell Signaling Research and Division of Molecular Life Sciences, Ewha Women's University, Seoul, Korea
[2] Department of Allergy and Rheumatology, Ajou University Hospital, Suwon, Korea

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Summary

Introduction: Aspirin is a popular nonsteroidal anti-inflammatory drug, but some patients suffer from hypersensitivity to it. This prompted us to identify the factors or molecules related to these responses.

Materials and Methods: A commercially available DNA microarray was used to study changes in gene expression in human peripheral blood mononuclear cells (PBMCs) after aspirin treatment. The PBMCs were collected from a patient with aspirin-intolerant asthma and one normal healthy control.

Results: We identified 61 and 107 genes respectively induced and repressed by aspirin treatment in the PBMCs derived from the normal control. In the patient showing aspirin-induced asthma responses, 31 genes were up-regulated and 6 were down-regulated after aspirin treatment. Among these, 1 gene was expressed with the same pattern in the control and the patient. In contrast, 19 genes showed different expression patterns, and it turned out that most of them were involved in immune responses, cell growth/proliferation, transcription/translation, and signaling pathways.

Conclusions: These results show the molecules involved in hypersensitivity to aspirin and may lead to a better understanding of adverse responses to aspirin. Furthermore, they can provide clues for identifying novel therapeutic and/or preventive molecular targets of the adverse effects of aspirin.

Key words: **aspirin • asthma • human peripheral blood mononuclear cells • microarray**

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Author's address: Prof. Kyunglim Lee, M.D. Ph.D., College of Pharmacy, Center for Cell Signalling Research and Division of Molecular Life Sciences, Ewha Woman's University, Seoul, Korea, tel.: +82 2 3277 3024, fax: +82 2 3277 2851, e-mail: klyoon@ewha.ac.kr

INTRODUCTION

Aspirin and other agents characterized as nonsteroidal anti-inflammatory drugs (NSAIDs) were designed primarily to decrease pain and inflammation. Since it inhibits platelet gathering and coagulation, aspirin is also used to prevent myocardial and cerebral infarction. Recently, many groups have reported that NSAIDs can suppress the growth of cancer cells from various organs by inducing the cells to cell-cycle arrest or apoptosis[1, 2, 4]. Aspirin and other NSAIDs can precipitate adverse reactions in two apparently different clinical conditions: bronchial asthma and chronic idiopathic urticaria. Although rare in children, this syndrome may occur in 20–25% of middle-aged patients with asthma, nasal polyps, or chronic urticaria, and can occur when these patients receive even small amounts (<80 mg) of aspirin. In some cases, these adverse effects can provoke a life-threatening reaction, but the underlying mechanism for these hypersensitivity reactions to NSAIDs is not well known[8].

With previous technologies, only a few candidate genes were identified and confirmed in different populations. These difficulties in identification highlight the importance of combining various approaches to increase our knowledge of the many pathways that are affected during the pathogenesis of aspirin intolerance and aspirin hypersensitivity-related genes. A relatively new method for the identification of molecular changes in response to certain chemicals and for drug-target validation is the DNA microarray[7]. Large-scale gene expression profiling with microarrays provides opportunities to investigate a large number of genes that may be implicated in the physiopathological processes that may occur when patients take aspirin.

Here we used a commercially available array to study the effects of aspirin on the peripheral blood mononuclear cells (PBMCs) of a normal person and an aspirin-sensitive patient with bronchial asthma. We compared the gene expression profiles of the two subjects and found 168 genes (in the normal person) and 37 genes (in the aspirin-sensitive person) that showed modified expression after aspirin treatment.

MATERIALS AND METHODS

Subject selection and characteristics

A 28-year-old male with aspirin intolerant asthma and a 36-year-old male as a normal healthy control were enrolled in this study. The former had typical asthmatic symptoms with a particular history of aspirin sensitivity and showed positive responses on lysine-aspirin bronchoprovocation tests. Their PBMCs were collected during the stable state of asthma after abstaining from anti--inflammatory medications for the previous 4 weeks.

PBMC preparation and aspirin treatment

Ten ml of peripheral venous blood was collected in an EDTA tube and PBMCs were purified using Ficoll--Paque (Pharmacia, Sweden). The number of viable cells was measured by tryphan blue dye exclusion assay. The cells (2×10^6 cells per well) were starved in DMEM media without serum for 5 h and incubated for 1 h with 50 μg/ml of lysine-aspirin (DongAh Pharmaceutical Co., Korea), which had proved to be the optimal concentration in a previous experiment[11], or the same amount of diluent as control.

RNA extraction and microarray studies

Total RNA was extracted from each culture using TRIZOL reagent (Life Technologies Inc., Rockville, MD, USA) according to the supplier's protocol and the yield was assessed by spectrophotometry. The microarray expression studies were performed using IntelliGene II Human CHIPs (Takara Bio Inc., Shiga, Japan). These arrays contain 3,893 cDNA fragments of identified human genes, including 979 kinds of cancer-related or cytokine-related genes, on a glass slide. To minimize technical variability, the RNA processing steps (RNA extraction, probe labeling, and chip hybridization) were performed in parallel for the control and asthmatic samples. Total RNA (1.5 μg) from aspirin-treated and untreated cells was labeled with Cy5-dCTP and Cy3-dCTP, respectively. The procedures for hybridization, washing, and detection of signal intensities have been previously described[9]. We used a 428 Array Scanner (Affymetrix) for scanning the chips and the scanned images were analyzed using ImaGene 4.2 (BioDiscovery). Signals were normalized between arrays using a correction factor calculated from the average expression of ten housekeeping genes. The genes having Cy5:Cy3 ratios greater than 2.0 or less than 0.5 were selected.

RESULTS

Of the 3,893 oligonucleotide probe pair sets contained on the IntelliGene II Human CHIPs, 1,120 and 1,048 probe sets were reliably detected in the PBMCs obtained from the control and the aspirin--sensitive subject, respectively. In the control, 168 known genes (217 transcripts) showed significantly different expression levels after aspirin treatment compared with pre-treatment. Of the genes, 61 (89 transcripts) were induced and 107 (128 transcripts) were repressed. 158 of these genes were grouped into 15 functional categories (Table 1). Sixteen genes

Table 1. Gene expression differences between pre- and post-aspirin treatment (in the normal control person)

Accession	Gene	Description	Fold change*	
			induction	repression
		Group 1: Immune signaling molecules		
NM_006463	STAMBP	molecule associated with the SH3 domain of STAM	2.60	
NM_000878	IL2RB	interleukin 2 receptor, beta	2.09	
NM_002185	IL7R	interleukin 7 receptor	5.10	
NM_000584	IL8	interleukin 8	3.58	
NM_018440	PAG	phosphoprotein associated with glyco-sphingolipid-enriched microdomains	3.15	
NM_002350	LYN	v-yes-1 Yamaguchi sarcoma viral-related oncogene homologue	2.30	
X60992	CD6	CD6 antigen		3.45
NM_004355	CD74	CD74 antigen (invariant polypeptide of major histocompatibility complex class II antigen-associated)		2.13
NM_001768	CD8A	CD8 antigen, alpha polypeptide (p32)		3.13
NM_000760	CSF3R	colony-stimulating factor 3 receptor (granulocyte)		4.00
NM_000206	IL2RG	interleukin 2 receptor, gamma (severe combined immunodeficiency)		5.56
NM_005356	LCK	lymphocyte-specific protein tyrosine kinase		2.50
NM_003682	MADD	MAP-kinase activating death domain		3.23
NM_002462	MX1	myxovirus (influenza) resistance 1, homologue of murine (interferon-inducible protein p78)		2.08
NM_000660	TGFB1	transforming growth factor, beta 1		2.94
NM_003331	TYK2	tyrosine kinase 2		3.13
		Group 2: Extracellular proteins		
NM_003246	THBS1	thrombospondin 1	2.04	
NM_007115	TNFAIP6	tumor necrosis factor, alpha-induced protein 6	2.33	
NM_000094	COL7A1	collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)		2.00
		Group 3: Immune response		
NM_004106	FCERIG	Fc fragment of IgE, high-affinity I, receptor for; gamma polypeptide		2.56
NM_006866	LILRA2	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2		2.13
NM_006674	HCP5	MHC class I region ORF		2.56
M12959	TRA@	T cell receptor alpha locus		3.23
		Group 4: Intracellular signaling		
NM_003567	BCAR3	breast cancer anti-estrogen resistance 3	3.15	
NM_018695	ERBB2IP	erbb2-interacting protein ERBIN	2.27	
NM_001496	GFRA3	GDNF family receptor alpha 3	2.29	
NM_006070	TFG	TRK-fused gene	2.23	
NM_006826	YWHAQ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	3.56	
NM_005451	PDLIM7	enigma (LIM domain protein)		11.11
NM_005248	FGR	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homologue		2.13
AB024574	GTPBP2	GTP-binding protein 2		3.03
NM_005273	GNB2	guanine nucleotide-binding protein (G protein), beta polypeptide 2		3.70
NM_005335	HCLS1	hemopoietic cell-specific Lyn substrate 1		2.33
NM_002110	HCK	hemopoietic cell kinase		2.50
NM_004517	ILK	integrin-linked kinase		5.88
NM_004635	MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3		2.56
NM_030662	MAP2K2	mitogen-activated protein kinase kinase 2		2.04
NM_002757	MAP2K5	mitogen-activated protein kinase kinase 5		2.70
NM_004834	MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4		2.13
NM_012392	PEF	PEF protein with a long N-terminal hydrophobic domain (peflin)		2.44
NM_002651	PIK4CB	phosphatidylinositol 4-kinase, catalytic, beta polypeptide		3.13
NM_001665	RHOG	ras homologue gene family, member G (rho G)		2.27
NM_005647	TBL1X	transducin (beta)-like 1		2.38
NM_005428	VAV1	vav 1 oncogene		2.22
BC010616	WBP2	WW domain binding protein 2		2.17

Table 1. cont.

Accession	Gene	Description	Fold change*	
			induction	repression
		Group 5: Proteolytic/antiproteolytic enzymes		
NM_001748	CAPN2	calpain 2, (m/II) large subunit	2.70	
NM_005213	CSTA	cystatin A (stefin A)	2.33	
NM_022739	SMURF2	E3 ubiquitin ligase SMURF2	2.74	
NM_012158	FBXL3A	f-box and leucine-rich repeat protein 3A	2.29	
NM_002816	PSMD12	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	2.39	
NM_005499	UBA2	SUMO-1 activating enzyme subunit 2	2.23	
NM_003336	UBE2A	ubiquitin-conjugating enzyme E2A (RAD6 homologue)	2.06	
NM_004788	UBE4A	ubiquitination factor E4A (homologous to yeast UFD2)	3.25	
NM_018693	FBX011	vitiligo-associated protein VIT-1	2.07	
NM_001749	CAPNS1	calpain, small subunit 1		3.03
NM_012483	GNLY	granulysin		3.13
NM_004994	MMP9	matrix metalloproteinase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase)		2.50
NM_002569	FURIN	paired basic amino acid cleaving enzyme (furin, membrane-associated receptor protein)		3.23
NM_002726	PREP	prolyl endopeptidase		2.08
NM_004716	PCSK7	proprotein convertase subtilisin/kexin type 7		2.04
NM_003064	SLPI	secretory leukocyte protease inhibitor (antileukoproteinase)		2.56
NM_003255	TIMP2	tissue inhibitor of metalloproteinase 2		2.44
		Group 6: Transmembrane proteins		
NM_014394	GHITM	growth hormone-inducible transmembrane protein	2.21	
NM_000876	IGF2R	insulin-like growth factor 2 receptor	2.59	
NM_002258	KLRB1	killer cell lectin-like receptor subfamily B, member 1	3.07	
NM_004475	FLOT2	flotillin 2		4.17
NM_002029	FPR1	formyl peptide receptor 1		2.70
NM_002205	ITGA5	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)		3.85
NM_005601	NKG7	natural killer cell group 7 sequence		2.56
NM_005608	PTPRCAP	protein tyrosine phosphatase, receptor type, C-associated protein		4.76
NM_002999	SDC4	syndecan 4 (amphiglycan, ryudocan)		3.13
AK027793	TM4SF8	tetraspan 3		2.00
		Group 7: Redox		
NM_006332	IFI30	interferon, gamma-inducible protein 30		2.17
AF258341	POR	P450 (cytochrome) oxidoreductase		3.45
		Group 8: Transcription/translation		
NM_014670	BZW1	basic leucine-zipper protein BZAP45	2.14	
NM_001271	CHD2	chromodomain helicase DNA binding protein 2	2.31	
NM_004396	DDX5	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68 kDa)	2.12	
NM_003472	DEK	DEK oncogene (DNA binding)	3.33	
NM_003908	EIF2S2	eukaryotic translation initiation factor 2, subunit 2 (beta, 38 kDa)	2.03	
NM_006037	HDAC4	histone deacetylase 4	2.21	
NM_014166	VDRIP	HSPC126 protein	2.38	
NM_002198	IRF1	interferon regulatory factor 1	2.98	
NM_022163	MRPL46	mitochondrial ribosomal protein L46	2.00	
NM_031419	MAIL	molecule possessing ankyrin repeats induced by lipopolysaccharide (MAIL), homologue of mouse	2.28	
U12767	NR4A3	nuclear receptor subfamily 4, group A, member 3	3.29	
NM_015317	PUM2	pumilio (Drosophila) homologue 2	2.92	
NM_033625	RPL34	ribosomal protein L34	2.90	
NM_002954	RPS27A	ribosomal protein S27a	2.04	
NM_001675	ATF4	activating transcription factor 4 (tax-responsive enhancer element B67)		2.04
NM_007273	REA	B cell associated protein		2.44
NM_023005	BAZ1B	bromodomain adjacent to zinc finger domain, 1B		2.27
NM_001421	ELF4	E74-like factor 4 (ets domain transcription factor)		2.38
NM_006732	FOSB	FBJ murine osteosarcoma viral oncogene homologue B		5.26
NM_007067	MYST2	histone acetyltransferase		2.17
NM_006084	ISGF3G	interferon-stimulated transcription factor 3, gamma (48 kDa)		3.70

Table 1. cont.

Accession	Gene	Description	Fold change*	
			induction	repression
		Group 8: Transcription/translation		
NM_005439	MLF2	myeloid leukemia factor 2		3.33
NM_006196	PCBP1	poly(rC)-binding protein 1		2.50
NM_002720	PPP4C	protein phosphatase 4 (formerly X), catalytic subunit		4.17
AJ243706	JARID1B	putative DNA/chromatin binding motif		2.33
NM_012448	STAT5B	signal transducer and activator of transcription 5B		2.17
NM_003244	TGIF	TGFB-induced factor (TALE family homeobox)		5.88
NM_006521	TFE3	transcription factor binding to IGHM enhancer 3		2.56
		Group 9: Cell growth and proliferation/apoptosis		
NM_001731	BTG1	B cell translocation gene 1, anti-proliferative	2.05	
AL132665	BNIP3L	BCL2/adenovirus E1B 19 kDa-interacting protein 3-like	2.83	
NM_006495	EVI2B	ecotropic viral integration site 2B	2.07	
NM_005109	OSR1	oxidative-stress responsive 1	2.02	
NM_020357	PCNP	PEST-containing nuclear protein	2.01	
NM_021127	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1	2.68	
NM_007217	PDCD10	programmed cell death 10	2.71	
NM_016085	C2orf28	apoptosis-related protein APR-3		2.04
NM_004656	BAP1	BRCA1-associated protein-1 (ubiquitin carboxy-terminal hydrolase)		3.70
NM_003993	CLK2	CDC-like kinase 2		2.63
NM_003992	CLK3	CDC-like kinase 3		2.76
NM_007065	CDC37	CDC37 (cell division cycle 37, *S. cerevisiae*, homologue)		2.27
NM_004394	DAP	death-associated protein		2.27
NM_004494	HDGF	hepatoma-derived growth factor (high-mobility group protein 1-like)		3.85
NM_014977	ACIN1	KIAA0670 protein/acinus		3.13
NM_005620	S100A11	S100 calcium-binding protein A11 (calgizzarin)		4.55
BC013082	SIAH2	seven in absentia (Drosophila) homologue 2		2.04
NM_003141	SSA1	Sjogren syndrome antigen A1 (52 kDa, ribonucleoprotein autoantigen SS-A/Ro)		2.22
NM_006342	TACC3	transforming, acidic coiled-coil containing protein 3		3.57
NM_004184	WARS	tryptophanyl-tRNA synthetase		2.63
NM_003288	TPD52L2	tumor protein D52-like 2		2.27
		Group 10: Cell adhesion molecules		
NM_002162	ICAM3	intercellular adhesion molecule 3		2.38
NM_004148	NINJ1	ninjurin 1		2.08
		Group 11: Metabolic enzymes		
NM_006886	ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	2.04	
AF084555	ARPP-19	cyclic AMP phosphoprotein, 19 kDa	2.02	
AL136939	ELOVL5	homologue of yeast long-chain polyunsaturated fatty acid elongation enzyme 2	2.01	
NM_005542	INSIG1	insulin-induced gene 1	2.00	
NM_000485	APRT	adenine phosphoribosyltransferase		2.13
NM_000034	ALDOA	aldolase A, fructose-bisphosphate		4.17
NM_000048	ASL	argininosuccinate lyase		3.03
NM_000858	GUK1	guanylate kinase 1		2.50
NM_012225	NUBP2	nucleotide-binding protein 2 (*E. coli* MinD like)		2.22
		Group 12: Cytoskeleton-related molecules		
NM_006135	CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1	2.59	
NM_006136	CAPZA2	capping protein (actin filament) muscle Z-line, alpha 2	2.33	
NM_021109	TMSB4X	thymosin, beta 4, X chromosome	3.13	
NM_006407	JWA	vitamin A responsive; cytoskeleton related	2.25	
NM_002018	FLII	flightless I (Drosophila) homologue		0.37
NM_007278	GABARAP	GABA(A) receptor-associated protein		0.43
NM_002859	PXN	Paxillin		0.22
NM_005022	PFN1	profilin 1		0.26
NM_003127	SPTAN1	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)		0.17
NM_006009	TUBA3	tubulin, alpha 3		0.45

Table 1. cont.

Accession	Gene	Description	Fold change*	
			induction	repression
		Group 13: Others involved in nucleic acid processing		
NM_031844	HNRPU	heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)	4.36	
NM_004593	SFRS10	splicing factor, arginine/serine-rich (transformer 2 Drosophila homologue) 10	2.20	
NM_007040	HNRPUL1	E1B-55 kDa-associated protein 5		3.13
		Group 14: Transport		
NM_002267	KPNA3	karyopherin alpha 3 (importin alpha 4)	2.34	
NM_004792	PPIG	peptidyl-prolyl isomerase G (cyclophilin G)	2.06	
NM_004047	ATP6V0B	ATPase, H+ transporting, lysosomal (vacuolar proton pump) 21 kDa		3.70
BC008861	ATP6V0D1	ATPase, H+ transporting, lysosomal (vacuolar proton pump), member D		4.00
NM_030673	SEC13L1	SEC13 (S. cerevisiae)-like 1		2.08
NM_013245	VPS4A	vacuolar sorting protein 4		2.86
NM_016830	VAMP1	vesicle-associated membrane protein 1 (synaptobrevin 1)		2.13
		Group 15: Chaperone/post-translational modification		
NM_001746	CANX	Calnexin		2.04
NM_000942	PPIB	peptidylprolyl isomerase B (cyclophilin B)		2.13
NM_002950	RPN1	ribophorin I		2.44

* Fold change:
– (expression level in post-aspirin treatment)/(expression level in pre-aspirin treatment) [in the case of induction]
– (expression level in pre-aspirin treatment)/(expression level in post-aspirin treatment) [in the case of repression]

were related to immune signaling molecules, 4 to immune response, 22 to intracellular signaling pathways, 17 to proteolytic enzymes, 21 to cell growth/proliferation, and 28 to gene transcription/translation. Other genes were involved in extracellular components (3), transmembrane proteins (10), redox (2), cell adhesion (2), and the cytoskeleton (10). In the aspirin-intolerant person (Table 2), 37 genes (42 transcripts) were significantly changed in expression level after aspirin treatment compared with pre-treatment. The expression levels of 31 genes (32 transcripts) were increased and those of 6 genes (10 transcripts) decreased. Thirty-six genes were classified into 12 groups. Three genes were associated with immune signaling molecules, 4 with immune response, 3 with intracellular signaling pathway, 3 with proteolytic enzymes, 3 with cell growth/proliferation, and 7 with gene transcription/translation. Other genes were related to transmembrane proteins (1), redox (1), and the cytoskeleton (5). In the control subject, more genes were down-regulated by aspirin treatment and repression fold changes were also higher. In contrast, more genes were up-regulated by aspirin in the person with asthma symptoms and, interestingly, most of these were shown to be repressed in the healthy person.

DISCUSSION

Asthma is a heterogeneous disease with variable clinical presentations. Airway inflammation and remodeling as well as immune responses are very important in the pathogenesis of asthma, and these are characterized by lymphocyte and eosinophil infiltration to the bronchial mucosa and are accompanied by structural changes. Both processes involve inflammatory and structural cells and likely play an important role in determining the development, chronicity, and severity of asthma[6].

In this study we investigated the global gene expression of PBMCs, which allowed a characterization of aspirin's effect on a normal and an aspirin-sensitive asthma patient. To find general patterns within the complex changes which occurred in a large number of genes, we grouped these genes into 15 functional categories. Most of the modified genes were related to immune response, cell growth/proliferation, intracellular signaling, and gene transcription/translation. More genes showed significant changes in expression level in the case of the control person. Different expression patterns between the control subject and the patient were observed in some genes (Table 3). They were down-regulated by aspirin treatment in the normal person and up-regulated in the aspirin-sensitive patient. The genes exhibiting this difference include FCEFIG[5, 12], CAPNS1[13], TYK2[3, 14], and TRA@[10], and these results are consistent with previously reported data showing that they can be candidate genes involved in asthma. In addition, expression pattern changes in the genes related to CD74, HCP5, HCK, other transcription-related molecules, and to cell proliferation were observed.

Table 2. Gene expression differences between pre- and post-aspirin treatment (in aspirin-sensitive person)

Accession	Gene	Description	Fold change	
			induction	repression
Group 1: Immune signaling molecules				
NM_001774	CD37	CD37 antigen	2.52	
NM_004355	CD74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class liantigen-associated)	2.18	
NM_003331	TYK2	tyrosine kinase 2	2.32	
Group 2: Extracellular proteins				
Group 3: Immune response				
NM_004106	FCERIG	Fc fragment of IgE, high-affinity I, receptor for; gamma polypeptide	2.48	
NM_019111	HLA-DRA	major histocompatibility complex, class II, DR alpha	2.06	
NM_006674	HCP5	MHC class I region ORF	2.26	
M12959	TRA@	T cell receptor alpha locus	2.49	
Group 4: Intracellular signaling				
NM_002110	HCK	hemopoietic cell kinase	2.11	
NM_002757	MAP2K5	mitogen-activated protein kinase kinase 5	2.01	
NM_006748	SLA	Src-like-adaptor	2.69	
Group 5: Proteolytic/Antiproteolytic enzymes				
NM_001749	CAPNS1	calpain, small subunit 1	2.13	
NM_002575	SERPINB2	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	2.20	
NM_033357	CASP8	caspase 8, apoptosis-related cysteine protease		3.03
Group 6: Transmembrane proteins				
NM_005601	NKG7	natural killer cell group 7 sequence	2.40	
Group 7: Redox				
NM_006332	IFI30	interferon, gamma-inducible protein 30	2.04	
Group 8: Transcription/translation				
NM_007273	REA	B cell-associated protein	2.16	
NM_001421	ELF4	E74-like factor 4 (ets domain transcription factor)	2.36	
NM_006732	FOSB	FBJ murine osteosarcoma viral oncogene homologue B	2.02	
NM_012423	RPL13A	ribosomal protein L13a	2.02	
NM_000992	RPL29	ribosomal protein L29	2.44	
NM_003472	DEK	DEK oncogene (DNA binding)		2.00
NM_020529	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha		2.13
Group 9: Cell growth and proliferation/apoptosis				
BC015055	FYN	FYN oncogene related to SRC, FGR, YES	2.02	
NM_005620	S100A11	S100 calcium-binding protein A11 (calgizzarin)	3.61	
NM_004184	WARS	tryptophanyl-tRNA synthetase	2.02	
Group 10: Cell adhesion molecules				
Group 11: Metabolic enzymes				
NM_000034	ALDOA	aldolase A, fructose-bisphosphate	2.45	
NM_005694	COX17	COX17 (yeast) homologue, cytochrome c oxidase assembly protein		2.04
Group 12: Cytoskeleton-related molecules				
NM_005022	PFN1	profilin 1	2.88	
NM_003127	SPTAN1	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	2.14	
NM_006009	TUBA3	tubulin, alpha 3	2.01	
NM_003370	VASP	vasodilator-stimulated phosphoprotein	2.15	
NM_003380	VIM	Vimentin		2.78
Group 13: Others involved in nucleic acid processing				
NM_194247	hnRNPA3	heterogeneous nuclear ribonucleoprotein A3		2.08
AL133623	SEP	similar to mouse Xrn1/Dhm2 protein		2.17
Group 14: Transport				
NM_001679	ATP1B3	ATPase, Na+/K+ transporting, beta 3 polypeptide	2.12	
Group 15: Chaperone/post-translational modification				

Table 3. The list of the genes that showed different expression patterns between the healthy person and the aspirin-induced asthma patient

Gene	Description
ALDOA	aldolase A, fructose-bisphosphate
REA	B cell-associated protein
CAPNS1	calpain, small subunit 1
CD74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)
ELF4	E74-like factor 4 (ets domain transcription factor)
FOSB	FBJ murine osteosarcoma viral oncogene homologue B
FCERIG	Fc fragment of IgE, high-affinity I, receptor for; gamma polypeptide
HCK	hemopoietic cell kinase
IFI30	interferon, gamma-inducible protein 30
HCP5	MHC class I region ORF
MAP2K5	mitogen-activated protein kinase kinase 5
NKG7	natural killer cell group 7 sequence
PFN1	profilin 1
S100A11	S100 calcium-binding protein A11 (calgizzarin)
SPTAN1	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)
TRA@	T cell receptor alpha locus
WARS	tryptophanyl-tRNA synthetase
TUBA3	tubulin, alpha 3
TYK2	tyrosine kinase 2

Undeniably, the results obtained from microarrays need to be validated with other techniques (for example *in situ* hybridization and immunohistochemistry). Nevertheless, this study shows that PBMCs from healthy and asthmatic subjects display distinct expression profiles. These differences provide a global view of the physiopathological processes active in aspirin-induced asthma and may provide invaluable help in clarifying the natural history of asthma. These results may be extended to explore other asthma phenotypes and may allow us to define changes in the expressions of genes of particular pathophysiological or therapeutic interest, which in turn may lead to more appropriate therapeutic interventions for patients.

REFERENCES

1. Akashi H., Han H. J., Iizaka M. and Nakamura Y. (2000): Growth-suppressive effect of non-steroidal anti-inflammatory drugs on 11 colon-cancer cell lines and fluorescence differential display of genes whose expression is influenced by sulindac. Int. J. Cancer, 88, 873–880.
2. Castonguay A., Rioux N., Duperron C. and Jalbert G. (1998): Inhibition of lung tumorigenesis by NSAIDS: A working hypothesis. Exp. Lung Res., 24, 605–615.
3. Duan W., Kuo I. C., Selvarajan S., Chua K. Y., Bay B. H. and Wong W. S. (2003): Antiinflammatory effects of genistein, a tyrosine kinase inhibitor, on a guinea pig model of asthma. Am. J. Respir. Crit. Care Med., 167, 185–192.
4. Funkhouser E. M. and Sharp G. B. (1995): Aspirin and reduced risk of esophageal carcinoma. Cancer, 76, 1116–1119.
5. Gounni A. S., Lamkhioued B., Koussih L., Ra C., Renzi P. M. and Hamid Q. (2001): Human neutrophils express the high-affinity receptor for immunoglobulin E (Fc epsilon RI): role in asthma. FASEB J., 15, 940–949.
6. Laprise C., Sladek R., Ponton A., Bernier M. C., Hudson T. J. and Laviolette M. (2004): Functional classes of bronchial mucosa genes that are differentially expressed in asthma. BMC Genomics, 5, 21–30.
7. Marton M. J., DeRisi J. L., Bennett H. A., Iyer V. R., Meyer M. R., Roberts C. J., Stoughton R., Burchard J., Slade D., Dai H., Bassett D. E., Hartwell L. H., Brown P. O. and Friend S. H. (1998): Drug target validation and identification of secondary drug target effects using DNA microarrays. Nat. Med., 4, 1293–1301.
8. Mastalerz L., Setkowicz M., Sanak M. and Szczeklik A. (2004): Hypersensitivity to aspirin: common eicosanoid alterations in urticaria and asthma. J. Allergy Clin. Immunol., 113, 771–775.
9. Meiyanto E., Mineno J., Ishida N. and Takeya T. (2001): Application of fluorescently labeled poly(dU) for gene expression profiling on cDNA microarrays. Biotechniques, 31, 406–413.
10. Moffatt M. F., Traherne J. A., Abecasis G. R. and Cookson W. O. (2000): Single nucleotide polymorphism and linkage disequilibrium within the TCR alpha/delta locus. Hum. Mol. Genet., 9, 1011–1019.
11. Pierzchalska M., Mastalerz L., Sanak M., Zazula M. and Szczeklik A. (2000): A moderate and unspecific release of cysteinyl leukotrienes by aspirin from peripheral blood leucocytes precludes its value for aspirin sensitivity testing in asthma. Clin. Exp. Allergy, 30, 1785–1791.
12. Rajakulasingam K., Till S., Ying S., Humbert M., Barkans J., Sullivan M., Meng Q., Corrigan C. J., Bungre J., Grant J. A., Kay A. B. and Durham S. R. (1998): Increased expression of high affinity IgE (FcepsilonRI) receptor-alpha chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. Am. J. Respir. Crit. Care Med., 158, 233–240.
13. Stewart A. G., Harris T., Fernandes D. J., Schachte L. C., Koutsoubos V., Guida E., Ravenhall C. E., Vadiveloo P. and Wilson J. W. (1999): Beta2-adrenergic receptor agonists and cAMP arrest human cultured airway smooth muscle cells in the G(1) phase of the cell cycle: role of proteasome degradation of cyclin D1. Mol. Pharmacol., 56, 1079–1086.
14. Tsang F., Choo H. H., Dawe G. S. and Wong W. S. (2002): Inhibitors of the tyrosine kinase signaling cascade attenuated thrombin-induced guinea pig airway smooth muscle cell proliferation. Biochem. Biophys. Res. Commun., 293, 72–78.