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Big data differential analysis of microglial cell responses in neurodegenerative diseases

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Abstract: Microarray technology has become an indispensable tool for monitoring the levels of gene expression in a given organism through organization, analysis, interpretation, and utilization of biological sequences. Importantly, preliminary microarray gene expression differs from experimentally validated gene expression. Generally, microarray analysis of gene expression in microglial cells is used to identify genes in the brain and spinal cord that are responsible for the onset of neurodegenerative diseases; these genes are either upregulated or downregulated. In the present study, 770 genes identified in prior publications, including experimental studies, were analyzed to determine whether these genes encode novel disease genes. Among the genes published, 340 genes were matched among multiple publications, whereas 430 genes were mismatched; the matched genes were presumed to have the greatest likelihood of contributing to neurodegenerative diseases and thus to be potentially useful target genes for treatment of neurodegenerative diseases. In protein and mRNA expression studies, matched and mismatched genes showed 99% and 97% potentiality, respectively. In addition, some genes identified in microarray analyses were significantly different from those in experimentally validated expression patterns. This study identified novel genes in microglial cells through comparative analysis of published microarray and experimental data on neurodegenerative diseases.

Key words: Microarray analysis, Microglia, Neurodegenerative diseases, Big data, Genes, Big data

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Introduction

Microarray analysis is a high-capacity system to monitor the expression levels of many genes simultaneously. Complementary DNAs on glass are used to quantitatively measure relative expression levels of corresponding genes; microarrays can be used to measure expression levels of thousands of genes in two or more tissue samples [1]. Although subsequent processes, such as differential degradation of mRNA in the cytoplasm and differential translation, are known to regulate gene expression, considerable knowledge can be gained by estimating relative quantities of mRNA species in populations of cells [2]. Recent studies have shown that resting and activated microglia show distinct phenotypes in the absence of nervous pathologies [3]. Activation of microglia by cerebral inflammation results in substantial loss of synaptic contact with adjacent neurons, suggesting a role for microglia in the initiation of synaptic stripping [4]. In addition, astrocytes exhibit biochemical and morphological changes in response to axotomy [5].

Microglial activation and proliferation in response to neurodegenerative diseases (Fig. 1) exert stimulatory effects through the dysregulation of chemical signaling using phagocytic products [6]. Evidence has accumulated regarding the role of microglia in altered synaptic remodeling, connectivity, and network functions, which contribute to the onset of

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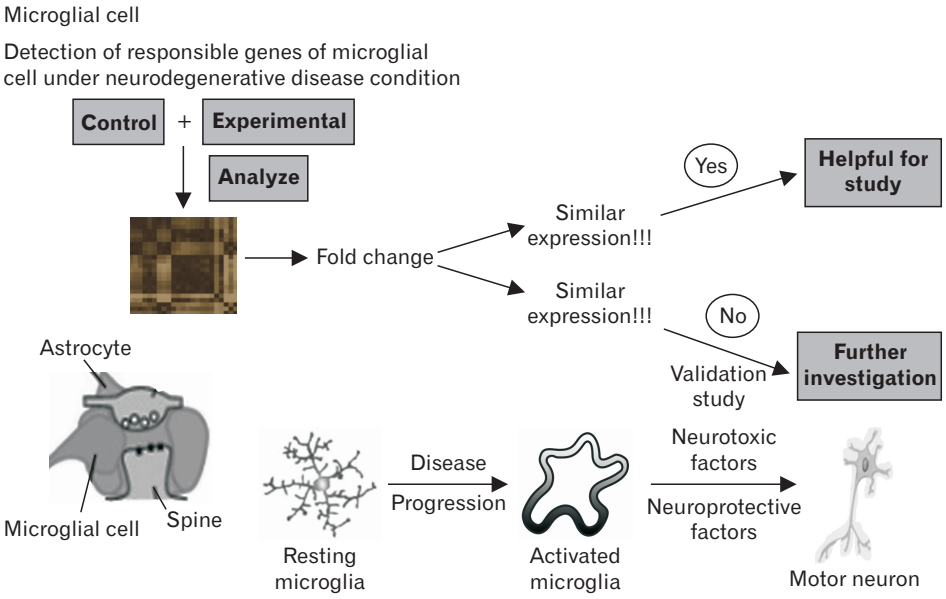

Fig. 1. Microarray study of microglial cell in neurodegenerative disease. This figure describes the activation of microglia is a hallmark of brain pathology. Under neurodegenerative disease condition, the inflammatory response is mediated by the activated microglia. This figure illustrates the gene expression pattern by microarray study in microglial cell under neurodegenerative diseases.

chronic pain-induced neurodegenerative disease; the underlying molecular and cellular mechanisms have become clear increasingly [7]. In addition, microglia have active roles in vital brain regions that are affected by memory-related aspects of neurodegenerative diseases. A previous overview of gene expression patterns in neurodegenerative diseases using microarray gene expression analysis provided information regarding major classes of transcriptional alterations observed in the cerebral cortex and cerebellum; these alterations were presumed to represent changes that occur throughout the central nervous system during aging [8]. In the neocortex and cerebellum, aging has been associated with activation of transcriptional responses in ways similar to those observed in human neurodegenerative disorders, such as the induction of lysosomal proteases, chaperones, and inflammatory factors [9]. The inflammatory response in the central nervous system is mediated by microglial activation and cytokine release, primarily including interferons and interleukins [10]. In Alzheimer's disease (AD), the responsible genes are calmodulin (*CALM3*), calnexin (*CANX*), AP-2 complex subunit beta (*AP2B1*), and apolipoprotein E (*APOE*). These genes have been shown by Cufflinks to exhibit significant splicing between normal and AD tissue in the whole brain, frontal lobes, and temporal lobes [11]. In spinal motor neurons in amyotrophic lateral sclerosis (ALS), the most upregulated gene is *hsa-mir-338-3p*, which showed a 12.7-fold change. This analysis of ALS enables to investigate cellular events that affect cell type-specific gene expression profiles in neurodegenerative disease [12]. In several research studies, spinal motor neurons and homogenates from ALS patients were analyzed, and cy5-labeled cDNA probes were synthesized from control samples. The data for each expressed gene obtained from microarray analysis were then expressed as the ratios of the values of individual ALS patients [12].

After nerve injury-induced neurodegeneration, microglia transform to reactive states through the gene expression of cell-surface receptors and proinflammatory cytokines [13]. Inhibitory factors and the expression of microglial molecules can strongly suppress a neurodegenerative disorder marked by tactile allodynia to mechanical stimuli. In patients with Sandhoff disease, microglial activation precedes acute neurodegeneration. Microarray gene expression profiling of patients with Sandhoff disease showed that cathepsin, serine protease inhibitor 2-1, metallothionein 1, glycoprotein 49A, and CD68 were highly expressed, with corresponding fold changes of 5.5, 4.9, 4.7, 4.5, and 4.5 [14]. Microarray gene expression profiling of patients with myoclonic epilepsy (EPMI) showed that *Wfdc17*, *lyz2*, *C3ar1*, *Mpeg1*, *ccl3*, and *Etnppl* were highly expressed, with corresponding fold change of 2.93, 2.28, 1.97, 1.75, and 1.73 [15].

In a recent gene microarray analysis, abundant transcripts for the proinflammatory cytokine osteopontin (*OPN*) were found in plaques of multiple sclerosis (MS) patients' brains [16]. *OPN* may participate in MS, as *OPN*-deficient mice were resistant to progressive experimental autoimmune encephalomyelitis [16]. The protein encoded by *OPN* is a potent modulator of autoimmune demyelination, and its expression in both neurons and microglia may influence neuronal degen-

eration in patients who are susceptible to MS [17]. *OPN* expression may also affect disease course and severity; increased expression levels of *OPN* have been found in the spinal fluid of MS patients during relapses [18].

This synthesis of microarray studies of microglial cell gene expression uses *in vitro* and *in vivo* sample data from published articles. In those published articles, differential gene expression levels in animal models were compared between control animals and animals exposed to experimental conditions of neuronal damage using microarray techniques; for the current study, fold-change values were acquired from published articles in PubMed and Web Med. In microarray studies, genes are classified according to upregulated and downregulated fold-change values. Here, genes are categorized as upregulated when their fold-change values are >1, and as downregulated when their fold-change values are <1. Genes are categorized as unchanged when their fold change values are equal to 1. Among the 770 total genes examined here, 456 were upregulated and 314 were downregulated.

In this study, published microglial cell gene expression microarray data are analyzed; preliminary microarray data are compared with experimentally validated data from real-time polymerase chain reaction (RT-PCR) or quantitative PCR (qPCR). This comparison does not entirely elucidate the responsible genes or particular characteristics of these genes in each disease. To identify genes of microglial cells that participate in disease, further validation is necessary using Western blotting (WB), immunohistochemistry (IHC), or *in situ* hybridization (ISH). The principal aim of this study was to identify novel genes and validate their expression levels in microglial cells using randomly selected studies of microarray data. The findings of this study can provide new insights into the origin and phenotypes of microglia in health and in multiple neurodegenerative diseases.

Materials and Methods

Listing of genes

Microarray gene expression levels of microglial cells were investigated, and 770 genes were identified by searching scientific articles that included analysis of microglial cells. The identified genes were compiled in a raw data file with some associated characteristics and important references. The control and experimental data were used to arrange the list of genes based on fold change; these genes were either upregulated or downregulated. Subsequently, each gene was searched in NCBI/PubMed for validation studies to identify experimentally validated genes. The genes were arranged in a raw data file to identify genes in microglial cells that were not studied yet. This method allowed identification of genes in

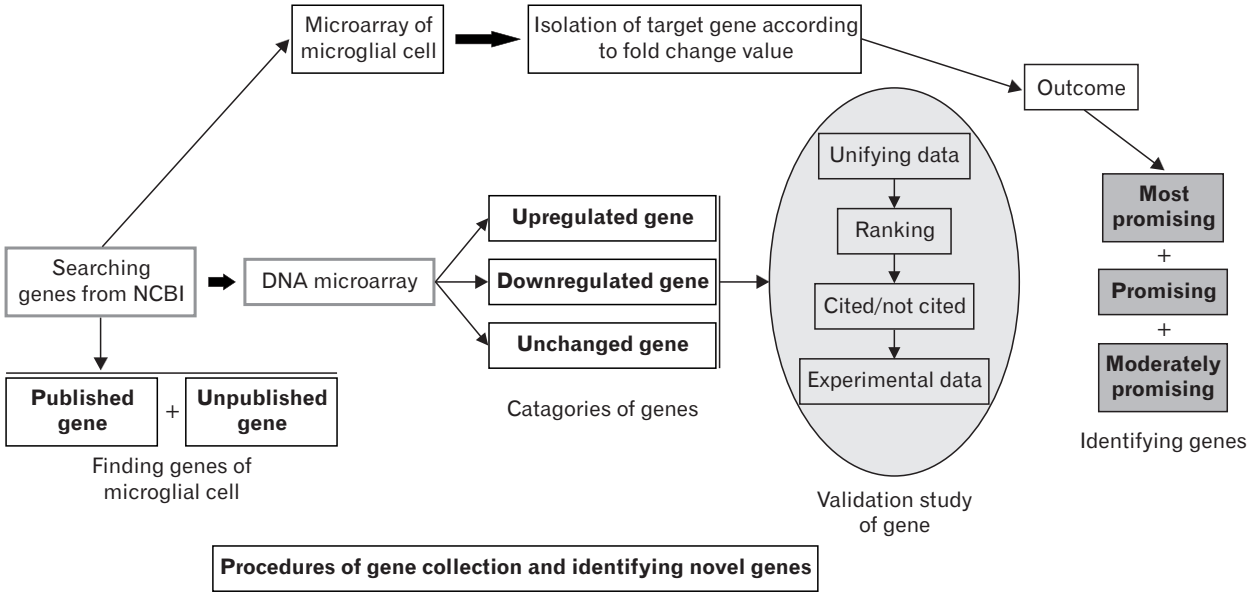

Fig. 2. Working procedure of finding various types of promising gene from microarray study. The procedure of searching genes of microglial cell study in the published research article regarding the DNA microarray to collect the data according to upregulated genes, downregulated genes as well as the unchanged genes. The novel genes are identified by microarray study. This illustration describes how each gene is searched in NCBI for collecting experimental data such as the polymerase chain reaction (PCR), real-time PCR, western blotting, or immunohistochemistry data. In this process, some target molecules are found from the microarray study.

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microglial cells for which changes in expression levels might contribute to neurodegenerative diseases. Fig. 2 shows the overall method for the study, included working procedures for gene collection and analysis. The procedure also included unification, as well as an assessment of the most promising, promising, and moderately promising genes.

Unification of fold-change values

Data were arranged in a spreadsheet in which upregulated genes had values of >1, and downregulated genes had values of <1; unchanged genes had values of 1. For example, the gene regulator of G-protein signaling 14 (*Abrcal*) [19] had a value of 30 in the microarray database, clearly indicating that it was an upregulated gene, whereas the gene nicotinamide adenine dinucleotide dehydrogenase 1 (*Ndufc1*) [20] had a value of 0.92 in the microarray database, indicating that it was a downregulated gene. During the collection of downregulated genes, the fold-change value of some genes was shown as a negative value (e.g., –1.25). In such cases, to unify the fold-change values, the negative inverse value was used (e.g., [–1/–1.25] or 0.8) to ensure that all downregulated genes had positive values <1.

Gene queries and gene ranking

In the raw data file, the genes were arranged according to their unified expression levels. For example, the gene with the highest value was *Inhbb*, which had a value of 72.6 and was located in the first row in the list. Subsequent genes were ranked by expression level, so that the gene with the lowest value, *Lefty*, with a value of 0.37, was located in row 770.

Cited and non-cited genes

Genes were then searched in PubMed. If we found no evidence of experimental work, such as qPCR, PCR, or WB, they were regarded as non-cited genes; if they had been investigated experimentally, they were regarded as cited genes. For example, the gene Bace2 had a microarray ranking of 753 with a fold-change value of 0.68; it had both *in vitro* and *in vivo* citations.

Gene searching for experimental data

Further searches of cited genes were performed to determine whether further experimental studies had been performed, such as RT-PCR, IHC, or ISH. Discrepancies between experimental data and microarray data were marked for additional analysis. For example, experimental data were found to validate the increased expression of the gene *Inhbb*, which had a microarray value of 72.6 [21].

Results

Positions of high- and low-ranked microarray genes

Microarray studies enable standardization of genome data, allowing target identification and facilitating drug discovery and development. Tables 1 and 2 show the genes with highest levels of upregulation and downregulation, respectively. Fig. 3 shows the mechanism by which microglial cells participate in neurodegenerative diseases through nerve injury and the activation of neuroinflammatory factors. Studies that previously would have included small numbers of samples now involve dozens or hundreds of assays. The current challenge is moving from data generation to data collection, management, and analysis to identify statistically and biologically significant

Table 1. Upregulated genes of microglial cell

No.	Gene name	Fold change	Regulation	Major function	Chromosome location	Experimental information	No. of references
1	*Inhbb*	72.6	Upregulated	Signaling protein	Chromosome 11	WB, PCR	1
3	*Pou3f*	71.7	Upregulated	Cell adhesion molecule	Not found	RT-PCR, IHC	2
71	*Arc*	62.4	Upregulated	Signaling protein	Chromosome 4	WB, IHC	2
98	*Htr5b*	56.5	Upregulated	Not found	Chromosome 3	RT-PCR, WB, IHC	2
261	*Rgs14*	38.1	Upregulated	Clearance of apoptosis	Chromosome 14	RT-PCR, IHC	1
361	*Cx3cr1*	30	Upregulated	Membrane protein	Chromosome 6	WB, PCR	1
363	*Tmem176a*	28.5	Upregulated	Cell adhesion molecule	Chromosome 7	RT-PCR, IHC	1
364	*Fxyd7*	23	Upregulated	Growth factor	Chromosome 1	Not found	1
365	*Rasl1 1b*	20.4	Upregulated	Transferase	Chromosome 5	WB, PCR	2+
403	*Fkbp1*	18	Upregulated	Not found	Chromosome 2	RT-PCR, WB, IHC	2

Inhbb, inhibin beta-B; WB, western blotting; PCR, polymeric chain reaction; *Pou3f*, pau class 3 transcription factor; RT-PCR, real-time PCR; IHC, immunohistochemistry; *Arc*, activity regulated cytoskeleton associated protein; *Htr5b*, 5-hydroxytryptamine; *Rgs14*, regulator of G protein signaling; *Cx3cr1*, chemokine receptor 1; *Tmem 176a*, transmembrane protein 176a; *Fxyd7*, Fxy domain containing ion transport regulator; *Fkbb1*, polyketide syntheses bb.

Table 2. Downregulated genes of microglial cell

No.	Gene name	Fold change	Regulation	Major function	Chromosome location	Experimental information	No. of references
456	*Sbk1*	0.88	Downregulated	Signaling protein	Chromosome 11	RT-PCR, IHC	2
463	*Ciao1*	0.82	Downregulated	Not found	Not found	WB, PCR	1
467	*Bcl11a*	0.71	Downregulated	Membrane protein	Chromosome 4	WB, IHC	2+
501	*Dbp*	0.69	Downregulated	Not found	Chromosome 3	WB, IHC	1
545	*Crybb1*	0.67	Downregulated	Clearance of apoptosis	Chromosome 14	RT-PCR, WB,IHC	2
559	*Fkbp1b*	0.62	Downregulated	Membrane protein	Chromosome 6	RT-PCR, IHC	2
570	*Htr5b*	0.53	Downregulated	Cell adhesion molecule	Chromosome 7	RT-PCR, IHC	2
600	*Gprc5b*	0.44	Downregulated	Growth factor	Chromosome 1	IHC	1
747	*Chtf8*	0.40	Downregulated	Not found	Chromosome 5	WB, PCR	1
770	*Lefty1*	0.37	Downregulated	Not found	Chromosome 2	RT-PCR, IHC	2

Sbk1, SH domain binding kinase 1; RT-PCR, real-time PCR; IHC, immunohistochemistry; *Ciao1*, cytosolic iron-sulfur protein assembly protein; WB, western blotting; PCR, polymerase chain reaction; *Bcl11A*, B-cell lymphoma 11A protein; *Dbp*, D site of albumin promoter; *Crybb1*, crystalline beta b1; *Fkbp1*, peptidyl-prolyl Cis-Trans isomerase; *Htr5b*, 5-hydroxytryptamine receptor 5B; *Gprc5b*, G protein-coupled receptor family C, group 5, member B; *Chtf8*, chromosome transmission fidelity factor 8 homolog (*Saccharomyces cerevisiae*); *Lefty1*, left right determination factor 1.

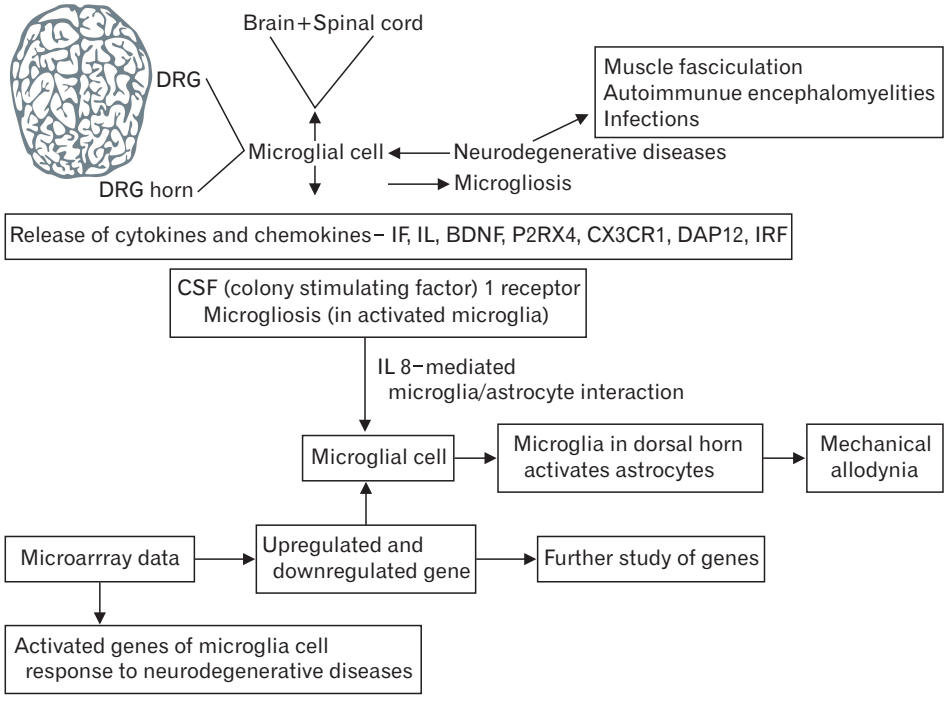

Fig. 3. Mechanism of microglial cell under neurodegenerative disease state. Neuronal cell death occurs under neurodegenerative disease state including motor and peripheral neuron. Microglial cell is activated when neuronal disease occurs. Microglia releases interferon (IF), interleukin (IL), brain-derived neurotrophic factors (BDNF), purinoreceptor (P2RX4), chemokine receptor 1 (CX3CR1), DAP12, and IRF when neuropathic pain as well as neurodegenerative disease occur. This diagram depicts how microglial cell becomes active under neurodegenerative diseases. DRG, dorsal root ganglia; CSF, cerebrospinal fluid.

patterns of gene expression. The highest microarray ranking in the left side of the graph indicates that these genes were highly upregulated, and the lowest microarray ranking in the right side indicates that these genes were highly downregulated (Fig. 4). Upregulated and downregulated genes were further classified based on whether they were supported by published experimental data separately, searchable in either PubMed or Google Scholar. Further stratification of the genes is shown in Fig. 4, wherein all upregulated genes are listed from 1 to 456 (Fig. 4A), and all downregulated genes from 457 to 770 (Fig. 4A). The overall distribution of upregulated and downregulated genes is shown in Fig. 4B: 456 genes were upregulated, and 314 genes were downregulated.

Classification of microarray genes according to experimental studies

Genes were further classified as type 1 (most promising genes), type 2 (promising genes), and type 3 (moderately promising genes) based on the number of experiments performed involving these genes. Type 1 genes were those for

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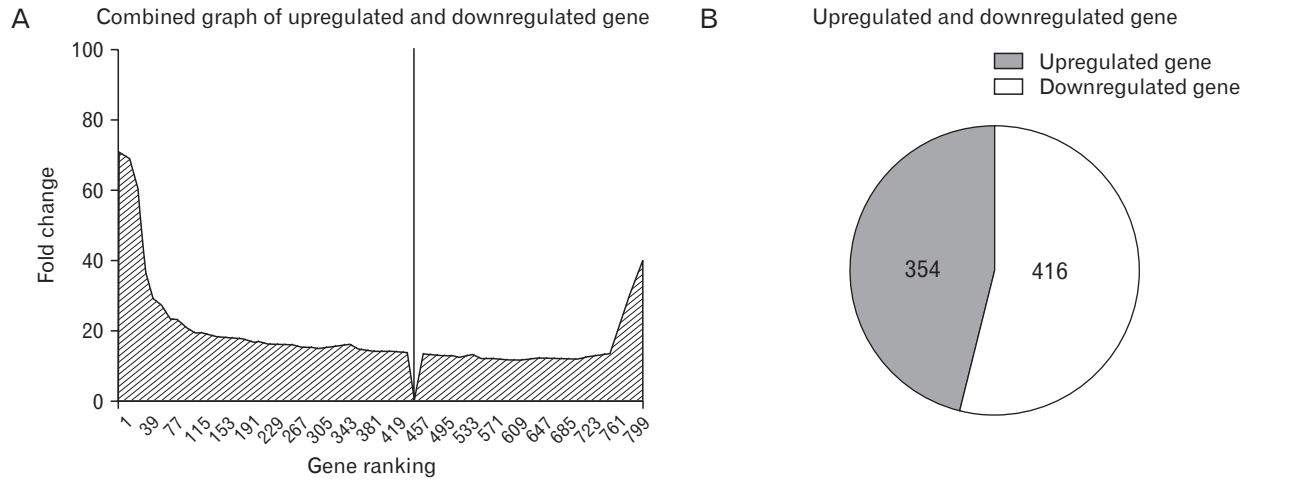

Fig. 4. Linear graph of upregulated and downregulated genes. (A) According to DNA microarray data, the overall numbers of total upregulated and downregulated genes found in the study which are studies related to microglial cell. Analyzing the total genes of upregulated and downregulated and the genes are searched for the experimental data such as polymerase chain reaction (PCR), real-time PCR, and quantitative PCR, etc. which indicates to find out possibility of some genes. (B) Illustration of the total amount of upregulated as well as downregulated gene. The upregulated genes are categorized when the fold-change value is >1 and the downregulated genes are categorized when the fold-change value is <1. The unchanged gene is marked when the fold-change value is 1. The total number of upregulated genes is 456 and the number of downregulated genes is 314 among the total 770 genes.

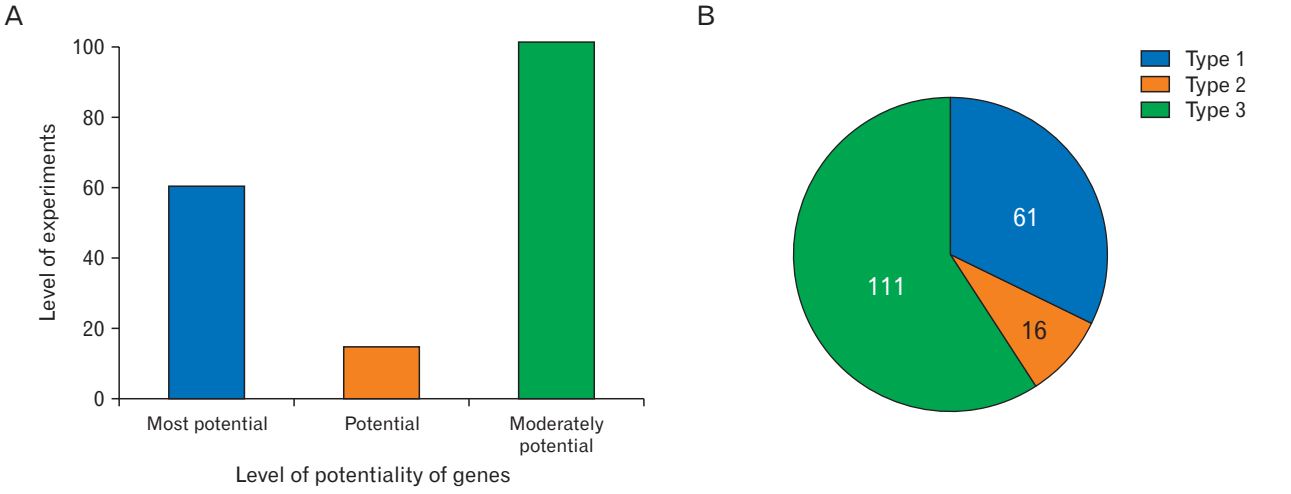

Fig. 5. Potentiality of gene compare to the experimental value. (A) According to the number of fold change, the upregulated and downregulated total genes are arranged in a unified way and according to the data, the genes are classified into most potential, potential, and moderately potential genes for identification. (B) Total amount of experimented genes compare to the published genes.The number of published genes is 770. Among them, 120 genes are most potential, 61 genes are potential, and 20 genes are moderately potential which are named as type 1, type 2, and type 3, respectively.

which protein levels were checked by WB and IHC; type 2 genes were those for which morphological expression levels were checked; type 3 genes were those for which mRNA expression levels were checked (Fig. 5). Genes were plotted in graphs (Fig. 5A) to show those with the greatest potential. The total numbers of articles found for type 1, type 2, and type 3 genes were 61, 20, and 120, respectively (Fig. 5B). Fig. 6 shows the microarray ranking compared to the percentage fold change, illustrating the total distributions of upregulated and downregulated genes in the study. The extent of experimental data found for each gene is indicated by dots of different colors, which can be compared to the blue dots that indicate microarray rankings in this study (Fig. 6A).

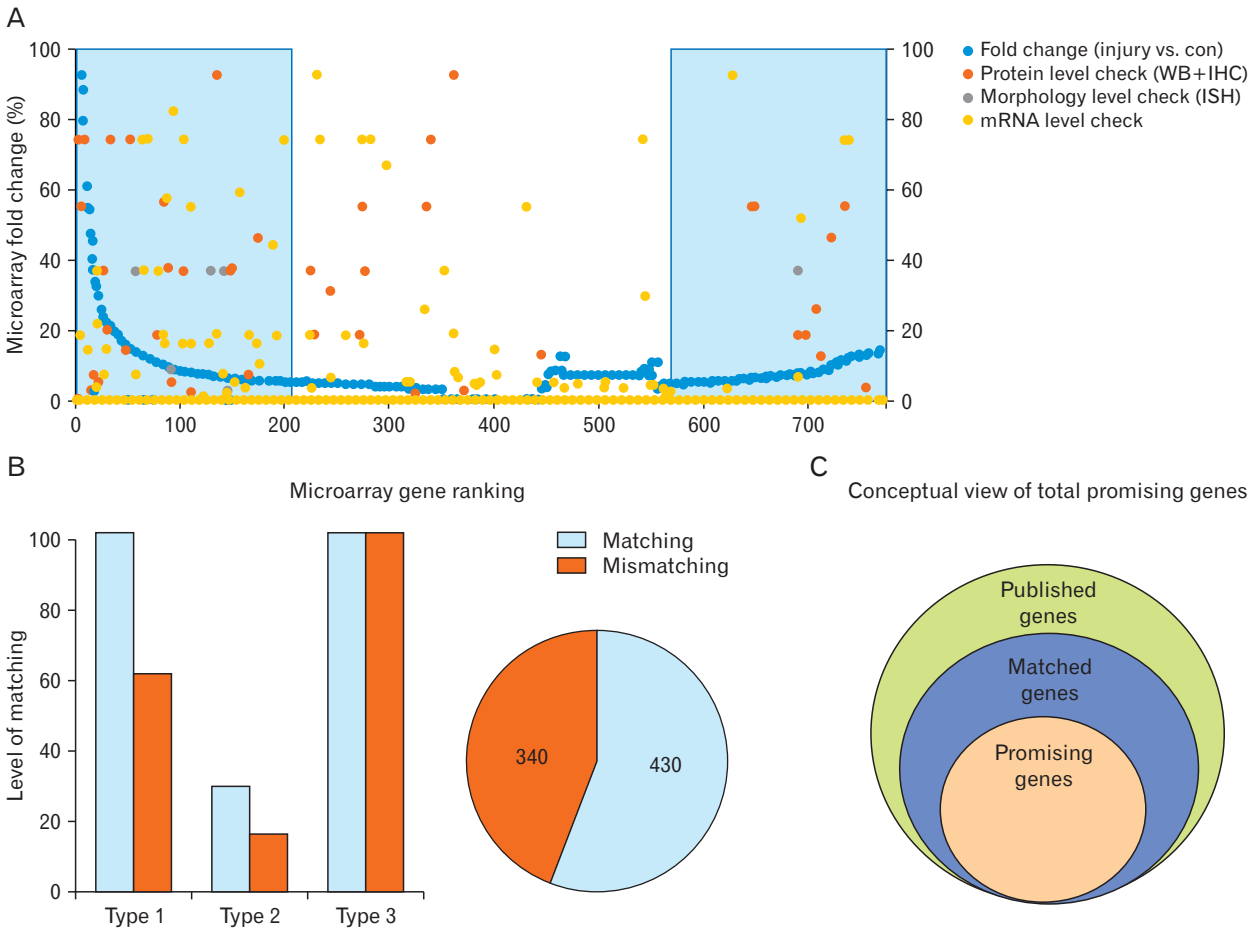

Fig. 6. Comparison between microarray gene expression and the real experimental value. (A) Total promising gene confirmation from the total published genes. As the left side of this graph shows these genes are highly upregulated and the right side of this graph shows highly downregulated, the blue marked area (both left and right) of both sides have good match and these are significant genes in this study. X-axis shows the total number of gene, n=770 and Y-axis shows the fold-change value. IHC, immunohistochemistry; ISH, *in situ* hybridization. (B) Differential range between the matched and mismatched gene. These figures show microarray data versus real experimental data such as western blotting (WB) and polymerase chain reaction (PCR)/quantitative PCR data. This figure shows the difference between experimental and microarray data. This graph also denotes the identification of find out highly promising gene in this study. (C) Differential range between the published, matched and promised gene. In the pie chart, the green color indicates published genes that occupied large space. Then the blue color indicates matched genes. The least number of promising genes are represented by the yellow color.

Summarized data of microarray genes by experimental studies

Microarray fold-change totals for matching and mismatching genes in this study were compared. As noted in the methods, matched genes were those for which microarray fold-change values indicated changes similar to those in experimental studies; mismatched genes were those for which microarray fold-change values showed no similarities in expression relative to those in experimental studies (Fig. 6). We identified 340 matched and 430 mismatched genes (Fig. 6A, B) in this study. Some microarray data showed lower expression levels in experimental studies, but higher expression levels in microarray studies. In Fig. 6B, dots that are close to the microarray ranking line indicate matched genes; these may be useful in further studies of microglial cell function. To summarize, the overall numbers of promising genes found and confirmed (Fig. 6C) included both upregulated and downregulated genes, marked in yellow (Fig. 6C); these exhibit potential for future studies of microglial cell function.

Discussion

Some microarray data showed expression levels that matched those in experimental studies; these could be useful

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in further studies of microglial cell function. In the analyses of all 770 published genes, we found that only 15% of all upregulated and downregulated genes were important for use in future microglial cell studies. Mismatched genes in this study were presumed to have no pathological role in disease. Moreover, matched genes were presumed to be useful as target genes in treatment of AD, ALS, MS, and EPMI neurodegenerative diseases. For example, the apolipoprotein gene (*APP*) was identified as a matched gene. This is consistent with the use of *APP* as the target gene for AD, and it has indeed been useful in elucidating the pathology of AD.

Microarray analysis of microglial genes can identify genes potentially responsible for nerve degeneration. This study included initial categorization of microarray results on microglial cell expression, as well as an arrangement of overall upregulated and downregulated genes. These genes were further categorized based on the extent of experimental studies found for each specific gene. Type 1 genes were those for which protein levels were checked by WB and IHC; type 2 genes were those for which morphological expression levels were checked; type 3 genes were those for which mRNA expression levels were checked. These experimental studies helped to establish the potential of the genes for use in future disease treatment based on the microglial cell response. This big data analysis established classifications of different kinds of genes and their characteristics and functions, such as upregulation or downregulation. In addition, some genes act differently based on experimental conditions, and this study provided further details regarding these genes. These findings may be useful for detection of gene expression in future experimental studies and thus for the proper diagnosis and treatment of diseases.

This study showed that some genes appear robust in microarray studies, but may not exhibit similar expression in experimental studies. Genes with matching data on expression from microarray analysis and experimental studies may be useful in further analyses of microglial cell function. Although no experimental methods were used in this study, the aggregated gene expression data in this study may enable further analyses of the microglial cell response to neurodegeneration.

Conclusion

Microarray analysis of genome arrays provides a robust, fully automated approach for examining gene function. This research helps to elucidate relationships among genes in neurodegenerative diseases and allows researchers to understand gene function based on expression patterns. Microarray technology provides a method for assessing the overall gene expression levels of microglial cells in neurodegenerative diseases. Among the experimental studies used as validation in this analysis, most included qPCR (mRNA expression) and WB (protein detection) techniques whose gene expression results matched the microarray data. Microarray analysis of a large number of genes has shown that the etiologies of pathological conditions may not always include novel genes. For gene screening, the method described here might enable prospective identification of genetic factors in microglial cells that contribute to neurodegeneration. To obtain additional microarray-derived lists of differentially expressed genes, further studies are necessary to increase the information available for biological interpretation.

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Conceptualization: RT, NYJ, HJC. Data acquisition: . Data analysis or interpretation: RT, NYJ, HJC. Drafting of the manuscript: RT, NYJ, HJC. Critical revision of the manuscript: NYJ, HJC. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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