Anakinra as an interleukin 1 receptor antagonist, complicated genetics and molecular impacts- from the point of view of mouse genomics

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Abstract

IL-1 receptor antagonist (IL-1rn) is a protein that binds to IL-1 receptors (IL-1r1) and inhibits the binding of IL-1α and IL-1β. In recent years, IL-1rn has been implicated to be associated with many human health problems. The effects of treatment of several inflammatory disorders with anakinra, which is an interleukin-1 (IL-1) receptor antagonist, have also been reported. Both positive and negative effects have been described. In this review, we systematically analyzed the expression, correlation, and regulation of IL-1rn and its 13 partner genes using available gene expression profiles from a variety of tissues in a well known transcriptome database, Genenetwork. The 13 partner genes include IL-1r1, IL-1β, IL-1α, Myd88, Irak1, Irak2, Irak4, Traf6, Tlr4, IL-1rap, Ikbkap, Nfkb1, and Nfkb2. Gene expression profiles are from 10 tissues including spleen, kidney, lung, whole brain, eye, prefrontal cortex, cerebellum, hippocampus, striatum, and nucleus accumbens. Our analysis indicated that the interactions among IL-1rn and its partner are complex and different from tissues to tissues, suggesting a broad spectrum of the effect of IL-1rn on biological and metabolic pathways. Transcripts and protein sequences resulted from different splicing, interaction with genomic background of individuals, and environmental factors affect function of IL-1rn. At present, our knowledge on the function of IL-1rn and its partner in various tissues or organs is very limited. The long term and extended effect of anakinra on human

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None.

health needs further investigations. In the future, targeted sequences or oligos of Il-1rn might be ideal for therapeutic application with less toxic and more specific in the treatment of specific disease. Detailed study on the molecular function of IL-1rn and its interaction with other genes and environmental factors is essential for development therapeutic application using IL-1rn.

Keywords

Anakinra; Arthritis; Expression; Genomic background; IL-1 receptor antagonist; Inflammation

1. Introduction

IL-1 receptor antagonist (IL-1rn) is a protein that binds to IL-1 receptors (IL-1r1) and inhibits the binding of IL-1α and IL-1β. Its expression level is known to increase in the blood of patients with a variety of infectious, immune, and traumatic conditions. IL-1rn was initially cloned and described in 1990 [1]. In human genomics its gene is located on chromosome 2, between 113,864,791 and 113,891,593 bps (according to Ensembl, Human GRCH37). It has 9 transcripts, including five with known protein sequences. In mouse, it is located on chromosome 2: between 24,192,373 and 24,207,014 bps (according to Ensembl, Mouse NCBIM37). It has 6 transcripts including 4 with known protein sequences. The largest protein sequence of mouse (ENSMUSP00000110126, with 178aa) is 87% similar to that of human IL-1RN isoform #3.

2. IL-1 receptor antagonist, human diseases, and pharmaceutics application

Shortly following its discovery in 1999, a number of human diseases or disorders has been linked to the polymorphism or expression of IL-1rn or its partners. Several chronic inflammatory diseases have been linked to polymorphism of IL-1rn in humans. Those human disorders include arthritis [2], systemic lupus erythematosus [3], ulcerative colitis [4], alopecia areata [5], multiple sclerosis [6], diabetic nephropathy [7], and fat mass [8].

Recent reports on linkage of Il-1rn with human diseases seem to increase its spectrum to many pathological and physiological problems. Those problems include neonatal onset of sterile multifocal osteomyelitis, periostitis, and pustulosis [9], neonatal onset of pustular rash, osteopenia, lytic bone lesions, respiratory insufficiency, and thrombosis [10], generalized aggressive periodontitis [11,12], refractory adult-onset Still's disease [13], coronary artery disease [14], schizophrenia [15], and risk of gastric cancer [16] and early stages of gastric carcinogenesis [17].

An important development related to research on function of IL-1rn is the clinic trial of anakinra, which is an interleukin-1 (IL-1) receptor antagonist. In a very recent period of time, it has been reported that it is effective on the treatment of several human inflammatory disorders, such as Mevalonate kinase deficiency (MKD)[18], systemic juvenile idiopathic arthritis (SJIA) [19–21], TNF receptor-associated periodic syndrome (TRAPS) [22], refractory chronic tophaceous gout [23], and refractory adult-onset Still's disease [13], Schnitzler syndrome [24], cryopyrin-associated periodic syndromes [25], refractory Sweet's

syndrome [26], autoinflammatory Muckle–Wells syndrome [27], persistent knee effusion [28], familial Mediterranean fever [29], Castleman's disease [30], and arthrofibrosis [31].

At the same time, at least several negative data or data suggesting precaution have been reported. Those data include the onset of focal bone erosion in neonatal-onset multisystem inflammatory disease [32], failure of anakinra treatment of pyoderma gangrenosum in an IBD patient [33], and hepatotoxicity due to tocilizumab and anakinra in rheumatoid arthritis [34]. Treatment with Anakinra improves disposition index but not insulin sensitivity in nondiabetic subjects with the metabolic syndrome [35].

These observations point out the complex role of IL-1rn in diseases and the function in human body. Those reports also suggest that IL-1rn may express or function differently in different tissues and IL-1rn and its partners may play different roles in the pathogenesis and pathophysiology in different tissues, as previously described by Luheshi and colleagues [36].

3. Expression levels of probes of IL-1rn and its partners in mouse tissues

To identify a group of genes that are relevant to IL-1rn, we input interleukin-1 receptor, type I; receptor for interleukin-1 alpha (IL-1A), beta (IL-1B), and interleukin-1 receptor antagonist protein (IL-1RA) on the Genenetwork (http://www.genenetwork.org/webqtl/main.py) [37], which consists of a set of linked resources for systems genetics. We then obtained the interaction partner genes of IL-1rn based on STRING analysis. As expected, as shown in Fig. 1, IL-1rn participate in different gene networks. Based on the Gene net work and our experience, we examined the expression level of 14 genes, Il-1r1, Il-1b, Il-1a, Myd88, Irak1, Irak2, Irak4, Traf6, Il-1rn, Tlr4, Il-1rap, Ikbkap, Nfkb1, and Nfkb2 and their interactions with each other. The expression levels in 10 tissues of those genes are from the same set of RI strain, the BXD strains. The combined BXD strain set at present is the largest RI mapping panel of mouse models (http://www.genenetwork.org/mouseCross.html#BXD). The BXD RI strains including 46 recently generated strains at Dr. Williams' laboratory [38] and 36 previously generated strains from the Jackson laboratory [39]. All of them are derived from C57BL/6J (B6) X DBA/2J (D2).

We examined the expression levels among IL-1ra and its partners in ten tissues, spleen, kidney, lung, whole brain, eye, prefrontal cortex, cerebellum, hippocampus, striatum, and nucleus accumbens. Microarray data are from Affymetrix M430.v2. The 430v2 array consists of 25-nucleotide probes that estimate the expression of genes and/or transcripts.

Table 1 lists the probes of each gene and their average expression levels in mice tested. Although numbers of mouse strains used in different tissues are different, all 14 genes express at a relatively high level across all 10 tissues. Among 14 genes, 10 have multiple probes: Il-1r1, Irak1, Irak4, Traf6, Il-1rn, Tlr4, Il-1rap, Ikbkap, Nfkb1, and Nfkb2. While the expression levels of different genes showed differences, majority of probes from the same gene also showed different levels too. 1) In general, probes from exons are different from probes from 3′ UTR. Particularly, the expression levels of two probes of Ikbkap, one at mid 3′ UTR while the other from exons, showed a significant difference ($P<0.0002$). One is at 10.18 while the other is at 7.61 (Table 1). Il-1rap has five probes from different location of

the gene. The expression level of probe 1449585 in exon region is much lower than that of other four probes, which are at the different distances of 3′ UTR. 2) The difference seems not only between probes from exons and 3′ UTR but also among probes from different positions from 3′ UTR. For example, there are three probes from Il-1rn, one is from the exons while the other two are from 3′ UTR. The two probes from 3′ UTR are from different positions, one is mid 3′ UTR while the other is from distal 3′ UTR. The expression levels of all three probes of Il-1rn are different one from the other. Irak1 has four probes from exons and 3′ UTR, all of them showed significant difference from each other. Four out of 6 probes of Traf6 showed significant difference from each other.

The universal expression and diversity pathways of IL-1rn and its partners among tissues and/or organs raise a critical issue on how a drug and/or molecule might work in human body. Current data accumulated in genenetwork suggest that IL-1rn and its partners are expressed in multiple tissues or organs, or perhaps they all are expressed universally in mouse or human body. Thus any drug or a targeted molecule that mimics the protein sequences or the function of IL-1rn will interact with genes in whatever tissues or organs that the drug or the molecule reaches to.

4. Relative expression levels of probes of genes among different tissues

The average expression levels of 37 probes of 14 genes in 10 tissues are similar except in the prefrontal cortex in which the expression levels of those probes are significantly lower than all other tissues. However, the absolute values of expression levels of genes in different tissues appear to have no effect on the relative expression levels among genes in the same tissues. To examine whether a relative expression level of a probe among all probes in different tissues are the same or not, we analyzed the correlation of gene expression among different tissues. Our analysis (Table 2) indicated that the expression levels among different tissue are from moderately positively correlated to highly positively correlated to each other. Among them, similar tissue types showed more positive correlated than non-similar tissues. For example, gene relative expression levels in whole brain, cerebellum, prefrontal cortex, and nucleus accumbens are highly correlated with the R of more than 0.9. While relative expression levels of probes in kidneys from male and female mice are near identical, with $R=0.9979$. There is a modest positive correlation between brain tissues (cerebellum, and prefrontal cortex, and nucleus accumbens) and internal organ tissues (kidney, spleen). The relative expression levels of genes in eye have a modest positive correlation with all other tissues, while the relative expression in lung has the lowest positive correlation with all other tissues.

In our examination of gene expression, correlation, and regulation, we used the same type of microarray chips of the same platform and the same set of RI strains derived from the same parental strains. Data from each of those studies are carefully inspected by investigators in each case (http://www.genenetwork.org/webqtl/main.py). Nevertheless, we realize that the number of strains used for different tissues or organs are different. Considering the fact that our data show the expression patterns of kidney tissues between female and male are nearly identical, and expression patterns from similar tissues are similar (such as tissues from brain), we feel confident of the conclusion from the analysis from those data.

5. Correlation of expression of probes among genes in different tissues

In order to examine whether the similarity of relative expression levels of probes in different tissues resulted a similar pattern in the gene–gene co-expression or correlation among probes of genes, we examined the correlation among probes of genes within tissues and compared their similarities. Because we have a total of 37 probes for the 14 genes, for each tissue, a total of 37×37 matrix Spearman Rank Correlations were produced from Genenetwork (Supplementary Table 1). Among those tissues, gene correlationships among kidney in male and female mice are measured separately, while others are predominantly female. The correlation among gene correlationship in those seven tissues shown in Table 3. As expected, gene correlationship of probes of genes from male and female kidney tissues are very correlated to each other, with R=0.72. However, in general, gene–gene co expressions are poorly correlated among of most of those tissues (Table 3). In particular, correlationship of genes derived from lung is at the weakest in relation to the correlationship of genes from other tissues, mostly with R equal or less than 0.1. The tissues from brain, including cerebellum, hippocampus, and whole brain have relatively higher R scores, between 0.2 and 0.3. Surprisingly, the gene correlation among striatum is independent of other tissues, including brain tissues, with R equal or less than 0.07.

One important note is that, while both positive and negative correlations among genes and/or probes are detected from 9 tissues, only positive correlations were shown from eye gene profiles of 71 RI strains.

Furthermore, because the molecular pathways and regulations of IL-1rn and its partners in different tissues are different from each other, the reaction with a drug or molecule in different tissues may trigger different molecular pathways, or result in the different phenotypes. Therefore, targeted specific tissue and accurate dosage in the drug application may be a key in the therapeutic application of a drug such as anakinra. Researches on delivery method and dosages seem to be essential for the drug development in the therapeutic application of drugs derived from of IL-1rn and its partners.

6. The potential influence of different splicing

The complications of interactions and regulations among Il-1rn and its partner genes at least partially due to the different splicing. For example, in Ensembl database (http://useast.ensembl.org/Mus_musculus/Info/Index), it listed 6 transcripts of mouse IL-1rn due to different splicing (Supplementary Table 2). While the Il1rn-001 and Il1rn-002 have a long (>1000 bp) 3′ end untranslated sequences, the other four have very short sequences at 3′ end. Although the tissue specificity of each transcript has not been clearly defined, the different expression levels detected by the three probes from IL-1rn may be a result of combination of expression levels of different transcripts in the different tissues. As tissue specific splicing has become known as the important regulatory mechanism of gene function [40–42], detailed consideration in target design of a gene or a pathway seems a critical issue. Regarding to anakinra, its molecule is a recombinant, non-glycosylated version of human IL-1RA with 153 amino acids. In humans, according to Ensembl database, IL-1rn gene encodes for nine transcripts, five of them have protein sequences. The anakinra molecule is

larger than two and small than the other three predicted proteins sequences of IL-1RN in humans, according to Ensembl database. Thus, the anakinra likely represents the function of at least three isoforms of IL-1RN proteins in human body. Unfortunately there is a limited report of the detailed study on the expression and tissue specificity of isoforms in either humans or mice [43].

7. Potential transcriptome mapping of Il-1r1 and IL-1rn in variety tissues

In order to investigate whether different probes are regulated differently, we analyzed the transcriptome map of those two genes. Il-1r1 has two probes on the M430.v2 chip from the distal 3′ UTR, but those two probes showed a significant difference in expression levels in different tissues. IL-1rn has three probes on the chip, one from exons, one from mid 3′ UTR and another is from distal 3′ UTR. Expression levels of those three probes showed a significant difference to each other. Surprisingly, our analysis indicated that there is no similarity among tissues for the transcriptome map of those probes (data not shown). Furthermore, there is no similarity even in kidney between female and male mice. Fig. 2 shows the transcriptome maps of two probes of Il-1r1 and three probes of IL-1rn between female and male kidneys. For probes of Il-1r1, in male there are suggestive regulatory loci on chromosome 2, 8, 12, and 16 for the second probe, the probe ID 1448950, located on distal 3′ UTR at 40,372,447 on chromosome 1. In female, however, there are two loci on chromosomes 3 and 19 that regulate the first probe, ID 1459777_at which is located at 40,260,465 on chromosome 1. For the three probes of IL-1rn, in male mice, there is a significant locus on chromosome 17 which regulates the third probe, which is at distal 3′ UTR located 24,206,409 on chromosome 2. In female mice, there are three regulatory loci that reach to significant level (LOD 3). One is a locus on chromosome 3 that regulates the second probe, ID 1451798. The other two loci are on chromosome 10, one regulate probe #1, ID 1423017, while the other regulate the probe #3, ID 1425663. Thus, the transcriptome regulatory loci in the map show that regulatory mechanism for IL-1rn and its partners are different not only among tissues but also among probes of the same gene.

8. Implication from IL-1rn knockout mice

Mice deficient for the production of interleukin 1 receptor antagonist (IL-1Ra) protein due to deletion of the IL-1rn gene has also implicated a complicated story. The mouse model was first described by Horai and coworkers [44] as a model for spontaneous arthritis. At present, it shows that disorders in the deficiency of IL-1rn are influenced by genomic background as well as environment factors. BALB/c mice that are homozygous for the deficiency (BALB−/−) develop inflammation in the hind limbs with an incidence approaching 100% beginning at about 6 weeks of age. However, not all strains of mice are susceptible. Both C57BL and DBA/1 mice are resistant. Furthermore, development of spontaneous arthritis appears to be dependent on exposure to microbial components, because these mice do not develop arthritis in germ-free conditions [45]. Recently, it was reported that BALB−/− mice induced aortic valve inflammation as well [46]; while B6−/− mice promotes Spontaneous Femoral Artery Aneurysm Formation in Mice [47].

These findings indicate that the influence of IL-1rn on disease development is much dependent on individual's genomic background and environmental factors. In spite of the considerable amount of work on the knockout of IL-1rn in several strains including congenics [48], a systematic investigation of impact of IL-1rn deficiency in all available mouse strains probably will shine some light on the function of IL-1rn in different genomic background, and therefore will be beneficial to the application of IL-1rn in therapeutic treatment for human diseases.

9. Future prospective

Taking the advantages of rapid development of technologies in genomics and protein analyses, future study should be on detailed analysis of the function of IL-1rn isoforms and its interactions with genomic backgrounds and environmental factors. The complexity of therapeutic applications targeting on IL-1rn pathway comes from several levels. First, IL-1rn is expressed in multiple tissues. Second, it interacts differently with different genes or pathways, suggesting it may play different roles in multiple pathways. Third, it has different isoforms that may play different roles in different tissues or pathways. And fourth, it is influenced by genomic background and environmental factors. Because of the different splicing patterns of the IL-1rn gene, it is critical to understand the relationship between each isoforms of the IL-1RN in particular tissues and its function. Furthermore, genetic variability of IL-1rn and in its isoforms, e.g. SNP in human population may result a different response of individuals to the same drug that targets a particular isoform of IL-1rn.

The complex interaction and regulation of IL-1rn and its partners in a variety of tissues and organs suggest that there is a long way to go to elucidate the molecular mechanism of IL-1rn and its partners. It implies that they may be relevant to multiple functions of human body, depending on genomic backgrounds of individuals. In other words, they may relate to multiple diseases or disorders. The key issue is the role each of them plays in a specific disease or disorders is not completely understood. Thus what would be the impact of their expressions on a specific disease or disorder, promotion, aggregation, or suppression? Targeted transgenic and knockout animal models in specific tissue or organs appear valuable tools for this purpose. Further studies of the molecular function of IL-1rn and genome-environment interaction are necessary.

Furthermore, considering the complexity of expression and interaction among IL-1rn and its partners and with genomic background and environmental factors, targeted specific sequences or oligos of Il-1rn might be less toxic and more specific to individual disease. Treatment with antisense oligos, short peptides, siRNAs have been recognized as efficient tools in therapeutic application. Study of specific binding sites or the interaction site and biding domains in the Il-1rn gene and its isoforms of proteins may have great potential in the improvement of the current therapeutic application of anakinra.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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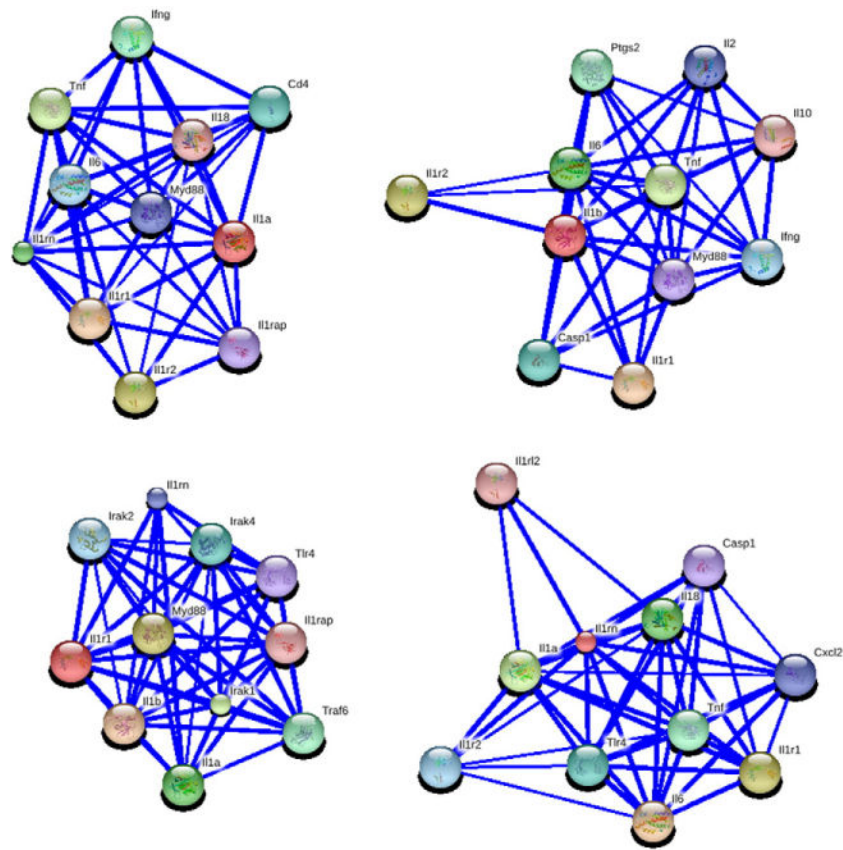
Fig. 1.
Potential gene network of IL-1rn. Data are generated using STRING 9.0. Nodes are colored
for predicted functional links. Stronger associations are represented by thicker lines.

Fig. 2.
Transcriptome maps of two probes of IL-1r1 and three probes of IL-1rn between female and male kidneys. Probes are from the M430.v2 chip of Affymetrix. The two probes of IL-1r1 are probe ID 1448950 and ID 1459777. The three probes of IL-1rn are ID 1451798, ID 1423017, and ID 1425663. KidneyM45=transcriptome mapping generated from gene expression profiles of kidneys of male mice from 45 BXD strains. Kidney F

65=transcriptome mapping generated from gene expression profiles of kidneys of female mice from 65 BXD strains.

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Mean expression level of IL-1rn and its partner genes.

Table 1

Probe ID	Gene symbol	Description	Location	Tissues											Everage	P value
				hippocampus	Cerebellum	Whole brain	Eye	Nucleus Accumbens	Prefrontal Cortex	Striatum	Kidney M	Kidney F	Spleen	Lung		
1451254_at	Ikbkap	inhibitor of kappa light polypeptide enhancer in B-cells, kinase complex-associated protein; mid 3′ UTR	Chr4: 56.763911	10.703	10.242	10.302	10.98	11.306	9.646	12.048	7.847	7.848	10.607	10.41		
1424142_at	Ikbkap	inhibitor of kappa light polypeptide enhancer in B-cells, kinase complex-associated protein; four exons (exons 26, 27, 28, 29)	Chr4: 56.800653	8.407	8.135	7.823	7.408	8.186	6.211	6.871	7.718	7.673	8.369	6.933	7.612182	0.000999
1421473_at	Il1a	interleukin 1 alpha; mid distal 3′ UTR	Chr2: 129.125444	6.21	5.912	6.732	6.565	5.664	3.761	6.651	6.426	6.422	7.676	7.286	6.300455	3.84E-05
1499399_a_at	Il1b	interleukin 1 beta; last exon and mid and proximal 3′ UTR	Chr2: 129.190645	7.634	7.745	7.323	6.563	7.437	5.6	7.33	8.068	8.002	9.625	9.238	7.687727	1.26E-06
1459777_at	Il1r1	interleukin 1 receptor, type 1; distal 3′ UTR	Chr1: 40.260465	6.001	4.807	4.285	5.134	4.681	3.073	5.311	5.69	5.677	5.456	5.29	5.036818	4.12E-07
1448950_at	Il1r1	interleukin 1 receptor, type 1; distal 3′ UTR	Chr1: 40.372447	8.367	8.441	8.676	9.17	8.071	6.785	6.553	9.51	9.469	9.969	10.28	8.663	8.34E-06
1449585_at	Il1rap	interleukin 1 receptor accessory protein	Chr16: 26.701452	6.054	5.424	4.848	5.59	5.745	3.672	6.791	6.154	6.275	6.245	7.149	5.813364	0.000133
1421843_at	Il1rap	interleukin 1 receptor accessory protein	Chr16: 26.716065	7.671	7.96	8.56	6.862	7.947	5.562	7.732	7.636	7.647	6.863	7.719	7.469	0.012041
1421844_at	Il1rap	interleukin 1 receptor accessory protein (hippocampal DG highly specific expression signature); far 3′ UTR	Chr16: 26.717242	8.03	8.181	9.037	7.731	8.589	5.689	8.957	7.301	7.366	7.912	8.811	7.964	0.02834
1442614_at	Il1rap	interleukin 1 receptor accessory protein	Chr16: 26.723185	8.628	7.365	8.205	6.725	7.63	7.202	7.49	6.224	6.194	6.291	5.83	7.071273	0.278097
1439697_at	Il1rap	interleukin 1 receptor accessory protein; far 3′ UTR	Chr16: 26.729648	8.884	7.8	8.772	7.398	7.501	7.274	8.474	5.959	5.976	5.731	5.786	7.232273	0.606911
1423017_a_at	Il1rn	interleukin 1 receptor antagonist; last three exons	Chr2: 24.203031	6.314	7.636	7.979	8.053	7.09	5.362	6.864	8.925	8.893	7.587	8.399	7.554727	0.003972
1451798_at	Il1rn	interleukin 1 receptor antagonist; mid 3′ UTR	Chr2: 24.205596	6.005	6.118	6.26	7.572	5.716	3.61	6.885	6.197	6.227	7.49	8.36	6.403636	0.017659
1425663_at	Il1rn	interleukin 1 receptor antagonist; distal 3′ UTR	Chr2: 24.206409	7.029	8.786	7.995	7.491	6.869	5.184	7.409	8.421	8.44	6.655	7.552	7.439182	3.06E-07
1460649_at	Irak1	interleukin-1 receptor-associated kinase 1; mid 3′ UTR	ChrX: 71.259671	8.866	10.236	10.056	9.216	8.891	7.212	10.025	10.678	10.46	10.431	9.912	9.634818	0.181412
1438120_x_at	Irak1	interleukin-1 receptor-associated kinase 1	ChrX: 71.260147	8.547	8.806	8.004	9.152	9.745	7.898	10.621	8.962	8.729	11.045	9.21	9.156273	0.001677
1438857_x_at	Irak1	interleukin-1 receptor-associated kinase 1	ChrX: 71.260147	7.861	8.668	7.722	8.296	8.414	6.262	9.388	8.829	8.707	8.987	7.882	8.274182	9.65E-05
1448668_a_at	Irak1	interleukin-1 receptor-associated kinase 1	ChrX: 71.260552	8.641	9.878	9.854	8.705	9.521	7.615	9.821	9.854	9.594	11.326	9.917	9.520545	0.010975
1436507_at	Irak2	interleukin-1 receptor-associated kinase 2; distal half of 3′ UTR	Chr6: 113.644249	8.01	9.369	9.186	9.642	8.948	7.061	8.929	9.385	9.386	10.154	9.054	9.011273	1.24E-08
1421670_a_at	Irak4	interleukin-1 receptor-associated kinase 4	Chr15: 94.384350	6.051	5.531	4.624	5.631	5.144	3.499	5.444	6.329	6.3	6.933	6.062	5.595273	0.000338
1451749_at	Irak4	interleukin-1 receptor-associated kinase 4	Chr15: 94.388763	5.868	6.341	6.261	6.627	6.566	4.201	6.47	6.796	6.74	8.724	7.658	6.568364	4.18E-05
1451750_at	Irak4	interleukin-1 receptor-associated kinase 4	Chr15: 94.398178	7.403	7.135	8.086	7.696	7.396	5.052	7.44	7.11	7.156	9.354	8.551	7.489	0.016957
1419272_at	Myd88	myeloid differentiation primary response gene 88	Chr9: 119.245183	7.776	7.116	7.386	8.462	7.222	5.537	8.413	9.119	9.151	10.395	10.34	8.265364	0.000276

Probe ID	Gene symbol	Description	Location	Tissues											Everage	P value
				hippocampus	Cerebellum	Whole brain	Eye	Nucleus Accumbens	Prefrontal Cortex	Striatum	Kidney M	Kidney F	Spleen	Lung		
1427705_a_at	Nfkb1	nuclear factor of kappa light chain gene enhancer in B-cells 1, p105; mid proximal 3′ UTR	Chr3: 135.247911	8.89	9.628	9.836	9.931	9.325	7.631	8.867	9.357	9.597	12.089	11.05	9.654909	4.1E-06
1442949_at	Nfkb1	nuclear factor of kappa light chain gene enhancer in B-cells 1, p105; intron 6	Chr3: 135.294808	6.171	7.281	7.099	6.846	7.392	5.218	5.806	7.234	7.192	6.746	6.167	6.650182	0.002332
1425902_a_at	Nfkb2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2, p49/p100; last three exons	Chr19: 46.385793	6.873	8.055	7.742	8.374	7.865	6.218	6.02	9.341	9.176	10.894	9.524	8.189273	0.060248
1429128_x_at	Nfkb2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2, p49/p100	Chr19: 46.386535	8.7	8.965	9.118	8.751	9.13	7.974	7.494	8.935	8.877	10.085	9.014	8.822091	4.8E-05
1418163_at	Tlr4	toll-like receptor 4; last exon and proximal 3′ UTR	Chr4: 66.502349	7.164	7.076	6.398	6.995	6.485	4.922	6.502	7.774	7.731	8.505	8.729	7.116455	0.000151
1418162_at	Tlr4	toll-like receptor 4; distal 3′ UTR	Chr4: 66.503254	6.082	4.889	4.754	6.785	5.158	3.347	6.096	6.138	6.22	7.883	8.314	5.969636	0.203133
1442827_at	Tlr4	toll-like receptor 4; far 3′ UTR	Chr4: 66.504146	6.157	5.273	5.11	5.8	5.297	3.465	6.024	5.882	5.893	6.62	7.725	5.749636	0.343018
1430695_at	Tlr4	toll-like receptor 4; deep 3′ UTR (alternative 3′ UTR?)	Chr4: 66.590773	6.47	5.633	5.252	5.391	5.021	3.366	5.727	6.33	6.311	5.638	5.468	5.509727	3.82E-05
1446940_at	Traf6	Tnf receptor-associated factor 6	Chr2: 101.525934	7.227	7.624	8.2	7.241	7.59	5.513	6.6	7.227	7.18	6.967	6.738	7.100636	0.755648
1421376_at	Traf6	Tnf receptor-associated factor 6; last exon and proximal 3′ UTR	Chr2: 101.537566	6.435	7.312	7.884	7.356	7.542	5.418	7.013	7.141	7.068	7.651	7.837	7.150636	9.22E-06
1421377_at	Traf6	Tnf receptor-associated factor 6	Chr2: 101.538253	5.835	6.554	6.374	6.389	6.352	4.148	5.777	6.722	6.678	6.592	7.132	6.232091	1.69E-05
1435350_at	Traf6	Tnf receptor-associated factor 6	Chr2: 101.541347	7.156	7.943	8.095	8.565	8.181	5.749	8.293	7.172	7.178	9.212	9.144	7.880727	0.000218
1443288_at	Traf6	Tnf receptor-associated factor 6	Chr2: 101.542176	6.868	6.513	6.706	6.699	6.716	4.324	7.896	6.506	6.483	6.277	7.456	6.585818	0.000378
1446977_at	Traf6	Tnf receptor-associated factor 6	Chr2: 101.542894	6.334	5.31	5.333	5.702	5.421	3.332	6.793	6.071	6.045	6.277	7.364	5.816545	2.65E-06

Table 2

Similarity in gene expression of IL–1rn and its partner genes among tissues.

	Hippocampus	Cerebellum	Whole brain	Eye	Nucleus accumbens	Prefrontal cortex	Striatum	Kidney M	Kidney F	Spleen	Lung
Hippocampus	1	0.829	0.830	0.763	0.864	0.924	0.811	0.560	0.565	0.589	0.508
Cerebellum		1.000	0.946	0.874	0.940	0.916	0.769	0.825	0.826	0.709	0.627
Whole brain			1.000	0.848	0.918	0.901	0.743	0.713	0.718	0.631	0.590
Eye				1.000	0.884	0.856	0.771	0.776	0.786	0.829	0.800
Nucleus accumbens					1.000	0.952	0.827	0.710	0.710	0.730	0.634
Prefrontal cortex						1.000	0.779	0.680	0.679	0.676	0.553
Striatum							1.000	0.530	0.530	0.595	0.585
Kidney M								1.000	0.998	0.813	0.747
Kidney F									1.000	0.816	0.762
Spleen										1.000	0.893
Lung											1.000

Table 3

Similarity of gene–gene interactions among tissues.

	Eye	Cerebellum	Lung	Kidney M	Whole brain	Hippocampus	Kidney F	nucleus accumbens	Prefrontal cortex	Striatum
Eye	1.000	0.266	0.244	0.159	0.191	0.216	0.157	0.125	0.175	−0.015
Cerebellum		1.000	0.020	0.271	0.279	0.396	0.230	0.152	0.123	−0.014
Lung			1.000	0.044	0.078	0.091	0.055	0.110	0.100	0.072
Kidney M				1.000	0.205	0.266	0.723	0.135	0.089	0.078
Whole Brain					1.000	0.299	0.149	0.197	0.316	0.045
Hippocampus						1.000	0.226	0.121	0.236	0.049
Kidney F							1.000	0.108	0.057	0.014
Nucleus accumbens								1.000	0.200	0.058
Prefrontal cortex									1.000	0.052
Striatum										1.000