Integration of major histocompatibility complex, methylation, and transcribed ultra-conserved regions analyses in uremia

Jinjun Qiu[1], Huiyan He[2], Weiguo Sui[3], Dong'e Tang[2] and Yong Dai[2]*

[1]Pingshan People's Hospital, Shenzhen, Guangdong, People's Republic of China

[2]Clinical Medical Research Center, The Second Clinical Medical College of Jinan University (Shenzhen People's Hospital), 518020, Shenzhen, Guangdong, People's Republic of China

[3]Nephrology Department of 181st Hospital, Guangxi Key laboratory of Metabolic Diseases Research, 541002, Guilin, Guangxi, People's Republic of China

Abstract

Treatment of uremia is now dominated by dialysis, in some cases, patients are treated with dialysis for decades, but overall outcomes are disappointing. A number of studies have confirmed the relevance of several experimental insights to the pathogenesis of uremia, but the specific biomarkers of uremia have not been fully elucidated. A total of 15 uremia patients and 15 healthy controls were collected in the present study. The aim of this study was to explain the etiology of uremia, MHC gene capture technology, hMeDIP-chip, T-UCR microarray and bioinformatics analysis were utilised in the uremia and normal control group. The result showed 8 CpG methylated enrichment in MHC segment. We found 1 SNP in CpG promoter of lncRNA and 1 SNP in chr6: 28890951-28892013, 1 SNP in CpG chr6:29521110-29521833 and 1 SNP in CpG chr6:30684836-30685503. The CpG methylated corresponding gene wasn't found significant immune correlated process GO term and KEGG pathway enrichment in uremia. In this experiment, T-UCR was not discovered in MHC segment. The T-UCR corresponding gene wasn't found significant immune correlated process GO term and KEGG pathway enrichment too. Analysis of SNP (rs2301754, rs11545587, rs17184255, rs4713354) and expression of the gene in peripheral blood lymphocytes indicated these SNP were associated with the occurrence of uremia. Future studies should examine the roles of these SNP in the pathogenesis of uremia. Integrative analysis technology provided an expansive view of molecular signaling pathways in uremia.

Introduction

Uremia refers to the condition that occurs when kidney function regresses during chronic kidney disease. Chronic kidney disease represents the progressive loss of renal function, and its latest stage-uremia, where little or no kidney function is present, requires either transplantation or dialysis [1]. In all stages of the disease, but particularly in uremia, patients present a many-fold increased mortality rate for cardiovascular disease than the general population. Despite intensive research, the pathologic mechanisms of uremia phenotype are still not completely understood and are probably multifactorial. Both genetic and environmental factors have been associated with uremia phenotype, but these factors cannot entirely explain the onset of uremia phenotype. Further studies are still encouraged to shed light on the true associations between uremia and its susceptibility genes. Novel methods should be looked into in this area.

Using a predictive bioinformatics algorithm, Mantila Roosa *et al.* created a linear model of gene expression and identified 44 transcription factor binding motifs and 29 miRNA binding sites that were predicted to regulate gene expression across the time course. Known and novel transcription factor binding motifs were identified throughout the time course, as were several novel miRNA binding sites. These time-dependent regulatory mechanisms may be important in controlling the loading-induced bone formation process [2]. This integrated bioinformatics analysis method may be looked into in our study. The link between MHC and uremia still unclear. Further investigations are likely to reveal the involvement of MHC in uremia. We are interested in studying MHC, CpG methylated and T-UCR as a first step toward better understanding regulation of gene expression in uremia. We report an expansive view of uremia from an integrated bioinformatics analysis of MHC, CpG methylated and T-UCR data sets.

Materials and methods

Human subjects

Thirty subjects were enrolled in the study including 15 uremia patients on dialysis and 15 healthy volunteers. All uremia patients were recruited from the inpatient unit in the Department of Nephrology in the181st Hospital and were free of active infections, diabetes mellitus, and autoimmune diseases.

Written informed consent was obtained from all the subjects or their guardians. The local Ethics Committee approved the study, and peripheral blood samples were obtained with informed consent from all

participating individuals. This study abides by the Helsinki Declaration on ethical principles for medical research involving human subjects.

MHC gene capture

Genomic DNA was isolated from the peripheral blood samples. According to the MHC genomic sequence, a completely complementary probe was designed and fixed on a support, and then applied to the genomic DNA after coupling with a probe connector. Unhybridized probe was washed away; then, probe that had hybridized with the DNA was eluted to directly build a library for DNA sequencing (Hiseq 2000 high-throughput sequencing). MHC region capture technology based on the NimbleGen SeqCap EZ Choice Library that enables deep sequencing coverage of the human MHC region. Data were analyzed by using the chi-squared test with Yates' correction for continuity.

hMeDIP-chip

Genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Fremont, CA). One microgram of the sonicated genomic DNA was used for immunoprecipitation using a mouse monoclonal antibody. For DNA labeling, the NimbleGen Dual-Color DNA Labeling Kit was used according to the manufacturer's guidelines that are detailed in the NimbleGen hMeDIP-chip protocol (NimbleGen Systems, Inc., Madison, WI, USA). The microarrays were hybridized in Nimblegen hybridization buffer/hybridization component A in a hybridization chamber (Hybridization System - Nimblegen Systems, Inc., Madison, WI, USA). For array hybridization, Roche NimbleGen's Promoter plus CpG Island Array was used.

T-UCR microarray analysis

Sample RNA labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) with minor modifications. The hybridized arrays were washed, fixed and scanned with using the Agilent DNA Microarray Scanner (part number G2505C). Agilent Feature Extraction software (version 11.0.1.1) was used to analyze acquired array images. Quantile normalization and subsequent data processing were performed with using the GeneSpring GX v12.1 software package (Agilent Technologies).

Bioinformatics analysis

MHC segment CpG methylated enrichment and difference enrichment analysis: MHC gene capture sequencing segment was chr6:30146860-33375560. To search enrichment location, we analyzed CpG peak in MHC segment.

T-UCR expression in MHC segment

To search transcript location, we analyzed T-UCR expression in MHC segment.

The effect of CpG methylated level and T-UCR expression level in immunologic process: We analyzed all methylated CpG, T-UCR and their corresponding gene. Then, we analyzed the related gene in immunologic process. In this experiment, to further understand the functions of the gene, we used the online gene ontology tool EASE (http://david.abcc.ncifcrf.gov/ease/ease1.htm. The differential expression gene was to classify in biological process. GO and KEGG pathway mapping of genes were performed by web-accessible DAVID annotation system.

The correlation of MHC mutation and CpG methylated

To search the correlation, we calculated data of differential CpG methylated, MHC mutation and analyzed correlation coefficient.

Results

Capturing the quantity of genes and SNP loci in the MHC region

We obtained 170 genes and 27,454 SNPs by MHC gene capturing and high-throughput sequencing in patients compared with the normal controls.

hMeDIP-chip: The 4063 genes of CpG islands showed significantly different methylation levels in the patients compared with the normal controls.

T-UCR microarray analysis: To identify potential T-UCRs differentially expressed, we performed a fold change filtering in the patients compared with the normal controls. There are 119 potential T-UCRs, which have been collected from authoritative databases such as Refseq, UCSC knowngenes, and Ensembl.

CpG peak in MHC segment

To search enrichment location, we analyzed CpG peak in MHC segment. The result showed 16 CpG methylated enrichment (Table 1), including 8 in uremia and 8 in the normal controls.

T-UCR expression in MHC segment

In this study, we analysis all the T-UCR expression, but UCR overlap gene and UCR proximal gene were not discovered in MHC segment.

The effect of CpG methylated level in immunologic process: In this experiment, we annotated CpG methylated corresponding gene with GO schemes by DAVID gene annotation tool. The genes produced total 55 GO terms in uremia (Table 2), and immune correlated process

Table 1. 16 CpG methylated enrichment in MHC segment.

CpG Name (hg19)	Length (bp)	Control	Uremia	Gene Name	Type
chr6:30038881-30039477	596		1	NCRNA00171	Promoter
chr6:30095173-30095610	437		1	TRIM40	Promoter
chr6:32046815-32047094	279		1	TNXB	Intragenic
chr6:32163292-32164383	1091		1	PBX2	Promoter
chr6:32163292-32164383	1091		1	GPSM3	Promoter
chr6:32847498-32847846	348		1	PPP1R2P1	Intragenic
chr6:33266302-33267582	1280		1	RGL2	Promoter
chr6:33266302-33267582	1280		1	WDR46	Promoter
chr6:28890951-28892013	1062	1		TRIM27	Promoter
chr6:29521110-29521833	723	1		UBD	Intragenic
chr6:30684836-30685503	667	1		MDC1	Promoter
chr6:30684836-30685503	667	1		TUBB	Promoter
chr6:31795467-31797384	1917	1		SNORD48	Promoter
chr6:31795467-31797384	1917	1		HSPA1B	Promoter
chr6:31795467-31797384	1917	1		C6orf48	Promoter
chr6:31795467-31797384	1917	1		SNORD52	Promoter

Table 2. The CpG methylated corresponding genes annotation GO terms in uremia.

GO Term	Gene Count	P Value	FDR
GO:0006350~transcription	378	8.17E-12	1.52E-08
GO:0006355~regulation of transcription, DNA-dependent	316	2.29E-09	4.25E-06
GO:0051252~regulation of RNA metabolic process	321	3.44E-09	6.39E-06
GO:0045449~regulation of transcription	436	4.54E-09	8.43E-06
GO:0007409~axonogenesis	55	3.41E-08	6.32E-05
GO:0030182~neuron differentiation	99	5.71E-08	1.06E-04
GO:0000904~cell morphogenesis involved in differentiation	64	7.04E-08	1.31E-04
GO:0048667~cell morphogenesis involved in neuron differentiation	57	9.74E-08	1.81E-04
GO:0006357~regulation of transcription from RNA polymerase II promoter	145	1.85E-07	3.44E-04
GO:0048812~neuron projection morphogenesis	56	4.70E-07	8.72E-04
GO:0048666~neuron development	77	1.62E-06	0.003011
GO:0006355~regulation of transcription, DNA-dependent	61	4.61E-06	0.008556
GO:0051252~regulation of RNA metabolic process	60	9.58E-06	0.017786
GO:0045449~regulation of transcription	58	1.00E-05	0.018585
GO:0007409~axonogenesis	61	1.83E-05	0.034300
GO:0030182~neuron differentiation	76	2.01E-05	0.037274
GO:0000904~cell morphogenesis involved in differentiation	36	2.52E-05	0.046809
GO:0048667~cell morphogenesis involved in neuron differentiation	39	5.75E-05	0.106653
GO:0006357~regulation of transcription from RNA polymerase II promoter	30	9.17E-05	0.170063
GO:0048812~neuron projection morphogenesis	80	9.86E-05	0.182883
GO:0048666~neuron development	75	1.12E-04	0.208311
GO:0031175~neuron projection development	46	1.31E-04	0.242746
GO:0032990~cell part morphogenesis	68	1.90E-04	0.351983
GO:0048858~cell projection morphogenesis	114	2.21E-04	0.409970
GO:0007389~pattern specification process	68	2.49E-04	0.461054
GO:0000902~cell morphogenesis	106	2.85E-04	0.528252
GO:0007156~homophilic cell adhesion	71	3.38E-04	0.625256
GO:0030900~forebrain development	116	3.41E-04	0.630759
GO:0006355~regulation of transcription, DNA-dependent	105	3.58E-04	0.662752
GO:0051252~regulation of RNA metabolic process	103	3.80E-04	0.703840
GO:0045449~regulation of transcription	106	3.93E-04	0.727092
GO:0007409~axonogenesis	87	4.04E-04	0.746483
GO:0030182~neuron differentiation	73	4.18E-04	0.772374
GO:0000904~cell morphogenesis involved in differentiation	103	4.68E-04	0.864433
GO:0048667~cell morphogenesis involved in neuron differentiation	60	4.69E-04	0.866098
GO:0006357~regulation of transcription from RNA polymerase II promoter	71	5.54E-04	1.022866
GO:0048812~neuron projection morphogenesis	93	6.35E-04	1.171092
GO:0048666~neuron development	60	6.70E-04	1.236425
GO:0031175~neuron projection development	94	7.01E-04	1.293726
GO:0032990~cell part morphogenesis	95	7.33E-04	1.351237
GO:0048858~cell projection morphogenesis	99	8.20E-04	1.510345
GO:0007389~pattern specification process	115	9.05E-04	1.666747
GO:0000902~cell morphogenesis	56	0.001007	1.853066
GO:0007156~homophilic cell adhesion	33	0.001143	2.099637
GO:0030900~forebrain development	87	0.001209	2.220594
GO:0007411~axon guidance	126	0.001287	2.362594
GO:0032989~cellular component morphogenesis	87	0.001377	2.525408
GO:0030030~cell projection organization	118	0.001636	2.992538
GO:0003002~regionalization	60	0.001677	3.066779
GO:0043009~chordate embryonic development	87	0.001789	3.269362
GO:0045935~positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	30	0.001931	3.523561
GO:0009792~embryonic development ending in birth or egg hatching	53	0.002101	3.828081
GO:0016192~vesicle-mediated transport	12	0.002437	4.427231
GO:0045892~negative regulation of transcription, DNA-dependent	12	0.002437	4.427231
GO:0051173~positive regulation of nitrogen compound metabolic process	118	0.002722	4.933267

Note: If p-value<0.005, it is considered significant. The False Discovery Rate (FDR) of a set of predictions is the expected percent of false predictions in the set of predictions. Meanwhile an FDR<5% might be quite meaningful.

GO term such as GO:0006955-immune response wasn't found significant enrichment.

In addition, we obtained 4 KEGG pathways of the genes in uremia (Table 3), immune correlated process KEGG pathway wasn't significantly enriched.

The effect of T-UCR expression level in immunologic process: In this experiment, we annotated T-UCR corresponding gene with GO schemes by DAVID gene annotation tool. The genes produced total 52 GO terms in uremia (Table 4), and immune correlated process GO term wasn't found significant enrichment. In addition, we don't obtained immune correlated process KEGG pathway of the genes in SLE. They weren't significantly enriched.

The correlation of MHC mutation and CpG methylated: In this experiment, we found 1 SNP in CpG promoter of lncRNA and 1 SNP in chr6: 28890951-28892013,1 SNP in CpG chr6:29521110-29521833 and 1 SNP in CpG chr6:30684836-30685503 (Table 5).

Discussion

The first genetic factors to be identified as important in the pathogenesis of uremia were those of the MHC on chromosome 6. It is now widely accepted that MHC genes constitute a part of the genetic susceptibility to uremia. Previous studies in uremia have lacked statistical power and genetic resolution to fully define MHC influences. In this research, we tried to identify MHC, CpG methylated and T-UCR, and reveal potential mechanism in uremia by a novel and combinatorial approach involving MHC gene capture technology, hMeDIP-chip, T-UCR microarray, and bioinformatic analysis. 27,454 SNPs were detected significantly different which may be involved in uremia. Moreover, in this study, we integrated the datasets and identified 4 most important SNPs in uremia. Function research on these SNPs is in our plan.

It is reported that H3K4me3 altered in uremia patients but not in healthy people [3]. Their results indicate that H3K9 trimethylation is involved in unphysiological uremic environment and these novel candidate genes may become potential biomarkers or future therapeutic targets [4]. Epigenetic events play a central role in the priming, differentiation and subset determination of T lymphocytes. CpG-DNA methylation and post-translational modifications to histone tails are the two most well accepted epigenetic mechanisms. The involvement of epigenetic mechanisms in the pathogenesis of uremia has been suggested A better understanding of the molecular events that contribute to epigenetic alterations and subsequent immune imbalance is essential for the establishment of disease biomarkers and identification of potential therapeutic targets. These findings may facilitate the selection of better target molecules for further studies. Our study might also aid in suggesting new pathways to be studied in a more focused approach with confirmation at the protein levels and investigation of the clinical significance.

Long non-coding RNAs (lncRNAs) are transcripts longer than ~200 nucleotides with little or no protein-coding capacity [5]. Growing evidence shows that lncRNAs present important function in development and are associated with many human diseases such as cancers, Alzheimer disease, and heart diseases. T-UCR transcripts are a novel class of lncRNAs transcribed from ultraconserved regions (UCRs). UCRs are a class of 481 noncoding sequences located in both intra- and inter-genic regions of the genome. UCRs are absolutely conserved (100%) between the orthologous regions of the human, rat, and mouse genomes, and are actively transcribed. It has recently been proven in cancer systems that differentially expressed T-UCRs could alter the functional characteristics of malignant cells. Recent data suggest that T-UCRs are altered at the transcriptional level in human tumorigenesis and the aberrant T-UCRs expression profiles can be used to differentiate human cancer types [6,7] . Researchers observed that DNA hypomethylation induces release of T-UCR silencing in cancer cells. The analysis of a large set of primary human tumors demonstrated that hypermethylation of the described T-UCR CpG islands was a common event among the various tumor types [8]. However, in our study, we integrated the MHC and T-UCR datasets. We examined the expression levels of T-UCR in MHC segment by T-UCR microarray. We annotated T-UCR corresponding gene by DAVID gene annotation tool. The immune correlated process GO term and KEGG pathway wasn't found significant enrichment. T-UCR expression levels were not correlated with commonly used clinicopathological features of uremia.

Conclusions

Taken together, we identified 4 most important SNPs (rs2301754, rs11545587, rs17184255, rs4713354) in uremia. Our work indicates that SNPs in MHC segment are potential biomarkers and probable factors involved in the pathogenesis of uremia. However, further studies are required to investigate the mechanism by which polymorphisms in this gene lead to uremia. A major advantage of combining multiple planes of measurement is the ability to dissect mechanisms not apparent in a single dimension. Integrating MHC, CpG methylated and T-UCR data sets is a powerful strategy for understanding uremia biology. Our findings proved insights into the anomalous regulated SNPs' potential contribution to the abnormalities in uremia and could help us to structure antenatal diagnostic biomarkers of uremia, as well as get the novel therapeutic targets in the treatment of individual with uremia. Besides, our study of SNPs may lead to finding novel methods to treat and prevent other diseases.

Acknowledgements

Table 3. The CpG methylated corresponding genes annotation KEGG pathways in uremia.

Pathways	Gene Count	P Value	FDR
hsa04012:ErbB signaling pathway	24	3.41E-04	0.418186
hsa05220:Chronic myeloid leukemia	20	0.001899	2.308109
hsa04144:Endocytosis	38	0.002299	2.787276
hsa05214:Glioma	17	0.004113	4.936479

Note: If p-value<0.005, it is considered significant. The False Discovery Rate (FDR) of a set of predictions is the expected percent of false predictions in the set of predictions. Meanwhile an FDR<5% might be quite meaningful.

Table 4. The T-UCR corresponding genes annotation GO terms in uremia.

GO Term	Gene Count	P Value	FDR
GO:0045449~regulation of transcription	240	8.45E-14	1.52E-10
GO:0051252~regulation of RNA metabolic process	176	1.11E-11	1.99E-08
GO:0006350~transcription	191	3.49E-10	6.27E-07
GO:0006357~regulation of transcription from RNA polymerase II promoter	87	4.20E-10	7.54E-07
GO:0006355~regulation of transcription, DNA-dependent	167	5.03E-10	9.05E-07
GO:0003002~regionalization	35	1.59E-08	2.85E-05
GO:0045941~positive regulation of transcription	66	1.74E-07	3.13E-04
GO:0045935~positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	70	3.44E-07	6.17E-04
GO:0010628~positive regulation of gene expression	66	5.15E-07	9.25E-04
GO:0051173~positive regulation of nitrogen compound metabolic process	71	5.44E-07	9.78E-04
GO:0016071~mRNA metabolic process	48	6.14E-07	0.001104
GO:0048598~embryonic morphogenesis	42	8.78E-07	0.001579
GO:0051254~positive regulation of RNA metabolic process	57	9.23E-07	0.001659
GO:0006397~mRNA processing	43	1.12E-06	0.002013
GO:0007389~pattern specification process	38	1.22E-06	0.002200
GO:0006396~RNA processing	62	1.30E-06	0.002335
GO:0045944~positive regulation of transcription from RNA polymerase II promoter	47	1.60E-06	0.002875
GO:0045893~positive regulation of transcription, DNA-dependent	56	1.60E-06	0.002876
GO:0048706~embryonic skeletal system development	18	2.01E-06	0.003616
GO:0008380~RNA splicing	39	2.10E-06	0.003773
GO:0009952~anterior/posterior pattern formation	25	2.31E-06	0.004161
GO:0031328~positive regulation of cellular biosynthetic process	72	2.61E-06	0.004690
GO:0048562~embryonic organ morphogenesis	24	3.21E-06	0.005772
GO:0010557~positive regulation of macromolecule biosynthetic process	69	3.84E-06	0.006907
GO:0009891~positive regulation of biosynthetic process	72	4.37E-06	0.007853
GO:0031327~negative regulation of cellular biosynthetic process	61	6.09E-06	0.010950
GO:0010604~positive regulation of macromolecule metabolic process	83	9.65E-06	0.017339
GO:0048568~embryonic organ development	27	9.77E-06	0.017567
GO:0030900~forebrain development	25	1.01E-05	0.018085
GO:0010558~negative regulation of macromolecule biosynthetic process	59	1.13E-05	0.020368
GO:0009890~negative regulation of biosynthetic process	61	1.17E-05	0.021059
GO:0010605~negative regulation of macromolecule metabolic process	72	2.79E-05	0.050223
GO:0001501~skeletal system development	39	3.18E-05	0.057209
GO:0010629~negative regulation of gene expression	53	6.67E-05	0.119750
GO:0021537~telencephalon development	14	9.53E-05	0.171182
GO:0051172~negative regulation of nitrogen compound metabolic process	52	2.63E-04	0.470982
GO:0021543~pallium development	11	2.97E-04	0.531764
GO:0016481~negative regulation of transcription	47	3.37E-04	0.603146
GO:0045934~negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	51	3.48E-04	0.623182
GO:0051253~negative regulation of RNA metabolic process	39	4.47E-04	0.800670
GO:0048704~embryonic skeletal system morphogenesis	12	4.50E-04	1.142592
GO:0045892~negative regulation of transcription, DNA-dependent	38	6.39E-04	1.268796
GO:0030902~hindbrain development	12	7.10E-04	1.636420
GO:0048705~skeletal system morphogenesis	17	9.18E-04	2.027251
GO:0000122~negative regulation of transcription from RNA polymerase II promoter	30	0.001139	2.820892
GO:0007411~axon guidance	16	0.001591	3.154926
GO:0030326~embryonic limb morphogenesis	14	0.001782	3.154926
GO:0035113~embryonic appendage morphogenesis	14	0.001782	3.206449
GO:0009954~proximal/distal pattern formation	7	0.001812	4.553220
GO:0009953~dorsal/ventral pattern formation	11	0.002589	4.620857
GO:0051051~negative regulation of transport	18	0.002629	4.877394
GO:0045665~negative regulation of neuron differentiation	8	0.002778	4.825061

Note: If p-value<0.005, it is considered significant. The False Discovery Rate (FDR) of a set of predictions is the expected percent of false predictions in the set of predictions. Meanwhile an FDR<5% might be quite meaningful.

Table 5. 4 CpG methylated SNPs of MHC segment in uremia.

Chromosome segment	SNP	Gene	Function
chr6: 30039098	rs2301754	RNF3	exonic
chr6: 28891522	rs11545587	TRIM27	UTR5
chr6: 29521289	rs17184255	LOC100507362	intergenic
chr6: 30685420	Rs4713354	MDC1	UTR5

Disclosure

The authors of this manuscript have no conflicts of interest to disclose.

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