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Ahmed Salim[1], Ihsan AlSaimary[1], Amal Adil Kasid Alsudany[2], Ahmed Salih Hussien Alshewered[3, 4] ✉
[1] College of Medicine, University of Basrah, Basrah, Iraq
[2] Basrah Health Directorate, Ministry of Health, Basrah, Iraq
[3] Misan Radiation Oncology Center, Misan, Iraq
[4] Misan Health Directorate, Ministry of Health/Environment, Misan, Iraq

Recording the New Genes for CCL2, CCL5 and CXCL10 Chemokines in Cases with Neuroinflammation and Multiple Sclerosis: A Brief Report

Conflict of interest: nothing to declare.
Authors' contribution: Ahmed Salim – conceptualization, data curation, investigation, methodology, project administration, resources, software, writing – original draft and editing; Ihsan AlSaimary – conceptualization, data curation, methodology, resources, writing – original draft and editing; Amal Alsudany – conceptualization, data curation, investigation, methodology, writing – original draft and editing; Ahmed Alshewered – conceptualization, data curation, investigation, methodology, resources, software, writing – original draft and editing.
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Contacts: ahmedsalihdr2008@yahoo.com

Abstract

Purpose. To discover new genes for CCL2, CCL5, and CXCL10 in patients with neuroinflammation and multiple sclerosis.
Materials and methods. The equipment and instruments used in this study with biological materials and chemicals used were from different countries and origins. The registered in NCBI under the accession of the GeneBank.
Results. New genes for CCL2, CCL5 and CXCL10 chemokines were identified, which were (LC727557), (LC727558) and (LC727558) respectively.
Keywords: multiple sclerosis, inflammatory neurological disease, CCL2, CCL5, CXCL10

Ахмед Салим[1], Ихсан АльСаймари[1], Амаль Адил Касид Альсудани[2], Ахмед Салих Хуссейн Альшеверед[3, 4]✉
[1] Колледж медицины, Университет Басры, Басра, Ирак
[2] Управление здравоохранения Басры, Министерство здравоохранения, Басра, Ирак
[3] Центр радиационной онкологии Мисана, Мисан, Ирак
[4] Управление здравоохранения Мисана, Министерство здравоохранения и окружающей среды, Мисан, Ирак

Выявление новых генов хемокинов CCL2, CCL5 и CXCL10 у пациентов с нейровоспалением и рассеянным склерозом: краткое сообщение

Конфликт интересов: не заявлен.
Вклад авторов: Ахмед Салим – концепция, обработка данных, проведение исследований, методология, ведение проекта, ресурсы, написание чернового варианта статьи, редактирование; Ихсан АльСаймари – концепция, обработка данных, методология, ресурсы, написание чернового варианта статьи, редактирование; Амаль Альсудани – концепция, обработка данных, методология, ресурсы, проверка подлинности полученных данных, написание чернового варианта статьи, редактирование; Ахмед Альшеверед – концепция, обработка данных, методология, ресурсы, программное обеспечение, написание чернового варианта статьи, редактирование.
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Подана: 15.04.2025
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Резюме

Цель. Выявление новых генов CCL2, CCL5 и CXCL10 у пациентов с нейровоспалением и рассеянным склерозом.
Материалы и методы. Оборудование и инструменты, использованные в данном исследовании, а также биологические материалы и химические вещества получены от разных производителей из разных стран. Результаты зарегистрированы в Национальном центре биотехнологической информации (NCBI) в разделе GeneBank.
Результаты. В результате исследования были идентифицированы новые гены хемокинов CCL2, CCL5 и CXCL10, получившие обозначения LC727557, LC727558 и LC727558 соответственно.
Ключевые слова: рассеянный склероз, воспалительное неврологическое заболевание, CCL2, CCL5, CXCL10

■ INTRODUCTION

Chemokines (chemoattractant cytokines) are small basic proteins (large group) with a molecular weight between (8–14 kDa) and are featured by attract leukocytes to the site of inflammation and infection [1].

Monocyte-derived neutrophil chemotactic factor (MDNCF), first discovered by Yoshimura et al. in 1987 [2]. Currently, this group has been extensively studied, and more than 50 different chemokines have been recorded in humen [3–6].

CCL2 (Monocyte Chemoattractant Protein-1): This chemokine is involved in recruiting monocytes to sites of inflammation. In MS, it plays a role in the migration of immune cells into the central nervous system (CNS), contributing to the inflammatory response. CCL5 (RANTES – Regulated upon Activation, Normal T cell Expressed, and Secreted): CCL5 is important in the recruitment of T cells, eosinophils, and basophils to inflammatory sites. Elevated levels are often observed in MS and other inflammatory diseases. CXCL10 (IP-10 – Interferon gamma-induced protein 10): This chemokine is strongly associated with Th1 responses and is involved in the attraction of T cells to inflammatory sites. CXCL10 is considered a critical factor in the pathogenesis of MS [7–9]. They play a role in immune regulation and T-cell polarization, induction of respiratory bursts, apoptosis, angiogenesis, mitosis, tumor metastasis, wound healing, and secretion of cytokines and extracellular matrix proteases. The main focus of MS chemokines is to gain further insight into lesion evolution, disease pathogenesis, and identification of potential therapeutic targets. However, definitive attributions of the pathogenic role of chemokines and receptors in human CNS diseases remain challenging [7]. Based on the knowledge about the diagnostic role of various chemokines that contribute to multiple sclerosis and the dynamic mechanism for its role in the early diagnosis, it was hypothesized that: Is a specific chemokine related to the pathogenesis of multiple sclerosis in Iraq, such as the presence of chemokines CCL2, CCL5, and CXCL10.

■ PURPOSE OF THE STUDY

To discover new genes for CCL2, CCL5, and CXCL10 in patients with neuroinflammation and multiple sclerosis.

■ MATERIALS AND METHODS

The equipment and instruments used in this study are listed in table 1.
The biological materials and chemicals used are listed in table 2.

Table 1
The equipment's and instruments used in the study

Item	Description and Company	Country
Butterfly Syringe	IMPROVE	China
EDTA tube	APCO	Jordan
Gel tube	Gongdong	China
Cold rack box	Biobasic	U.K.
Disposable glove	Care gloves	Malaysia
Centrifuge	NUVE	Turkey
Eppendorf Tube	1.5 ml, ABDOS	India
Disposable tips	20, 200, 1000 ml, Citotest	China
Micropipette	10–1000, Biobase	Germany
Horizontal electrophoresis system	Mupid-One	Japan
Gradient Thermal Cycler	T100 Thermal Cycler, BioRad	Singapore
Microcentrifuge	Mikro 120, Hettich	Germany
Vortex mixer	LVM-202, DAIHAN	Korea
Water Bath	LWB-111D, DAIHAN LabTech	Korea
Elisa Reader	Mindray	China
Distilled water	Alab Tech	Korea
Incubator	Memmert	Germany
Microwave Oven	Panasonic	Japan

Table 2
The chemical and already prepared solution

Item	Description and Company	Country
1500pb DNA ladder	Lot: 1101C, Cat. No. D-1030, Volume 250 µl, Concentration 135ng/ µl. Bioneer	Korea
10x TBE (Tris-Borate-EDTA) buffer 1 liter bottle	Bio Basic Inc.	Canada
Ethanol	J.K. Baker	Netherland
Agarose	Bio Basic Inc.	Canada
Bromophenol blue	Bio Basic Inc.	Canada
Ethidium bromide (10 mg/ml Solution)	Bio Basic Inc.	Canada
Nuclease free water	Bioneer	Korea

Ethics Statement

This study was approved by the IRB committee of the Research Units, Training and Humanity Development Center, Basrah Health Directorate, and Department of Medicine, University of Basrah/Researches Units, Training and Humanity Development Center, Basrah Health Directorate (No.109/2021 [479] on 17/11/2021 and No. 855 on 21/11/2021).

■ RESULTS

The newly identified genes are shown in figure 1, 2 and 3. The new genes for CCL2, CCL5 and CXCL10 chemokines were recorded, and the results were registered in NCBI under the accession numbers (LC727557), (LC727558) and (LC727558) respectively.

■ DISCUSSION

Depending on the no. and spacing of cysteine residues included in the formation of disulfide bonds, the chemokines are categorized into 5 groups, which are: C-C (β-chemokine), C-X-C (α-chemokine), X-C (δ-chemokine (C-subfamily)), C-X-3-C (γ-chemokine) and C-X chemokines [8–10].

The chemokines of C-C, C-X-C, and C-X-3-C families have 4 cysteines, X-C chemokines only have 2. C-C chemokines are the largest group containing two adjacent cysteine residues near their N-terminus, and genes are grouped on chromosome-17 in humen [4–6].

In the C-X-3-C and C-X-C chemokine subfamilies, there are 1 to 3 additional amino acids (represented 3X or X) separate the 1st two of the four cysteine residues, and most of the C-X-C chemokines are clustered on chromosome-4 in humans. The 5th sub-family C-X chemokine, which was recently identified in zebra-fish by Nomiyama in 2008, lacks one of the two N-terminal cysteine residues, but retain the 3rd and 4th [2, 10].

These new genes cannot be compared with other studies because no data were found in the literature.

Clinical Relevance

Play an important roles as prognostic biomarkers: The expression levels of chemokines in blood or CSF may serve as biomarkers for disease activity or progression in MS. In addition, the roles as therapeutic targets: Chemokine inhibitors or blockers targeting

GenBank ▾ Send to: ▾

Homo sapiens CCL2 gene for C-C motif chemokine ligand 2, partial sequence

GenBank: LC727557.1

FASTA Graphics

Go to:

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ACCESSION   LC727557
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            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
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REFERENCE   1
  AUTHORS   Amal,A.K., Ihsan,E.A. and Ahmed,A.S.
  TITLE     Immunopathogenesis, molecular determination and neuro inflammatory
            role of ccl2, ccl5 and cxcl10 chemokines among patients with
            multiple sclerosis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 442)
  AUTHORS   Kasid,A.A., Ihsan,E.A. and Ahmed,A.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (03-SEP-2022) Contact:Amal Adil Kasid Ministry of Higher
            Education and Scientific Research, University of Basra, College of
            Medicine, Microbiology; The Schools Street, Hay AL Hussain, Basrah,
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Fig. 1. Homo sapiens CCL2 gene for C-C motif chemokine ligand 2

GenBank ▾ Send to: ▾

Homo sapiens CCL5 gene for C-C motif chemokine ligand 5, intron 1, partial sequence

GenBank: LC727558.1

FASTA Graphics

Go to: ⊡

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ACCESSION   LC727558
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REFERENCE   1
  AUTHORS   Amal,A.K., Ihsan,E.A. and Ahmed,A.S.
  TITLE     Immunopathogenesis, molecular determination and neuro inflammatory
            role of ccl2, ccl5 and cxcl10 chemokines among patients with
            multiple sclerosis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 449)
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  JOURNAL   Submitted (03-SEP-2022) Contact:Amal Adil Kasid Ministry of Higher
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Fig. 2. Homo sapiens CCL5 gene for C-C motif chemokine ligand 5

GenBank ▾ Send to: ▾

Homo sapiens CXCL10 gene for C-X-C motif chemokine ligand 10, partial sequence

GenBank: LC727559.1

FASTA Graphics

Go to:

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ACCESSION   LC727559
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  ORGANISM  Homo sapiens
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            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Amal,A.K., Ihsan,E.A. and Ahmed,A.S.
  TITLE     Immunopathogenesis, molecular determination and neuro inflammatory
            role of ccl2, ccl5 and cxcl10 chemokines among patients with
            multiple sclerosis
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 273)
  AUTHORS   Kasid,A.A., Ihsan,E.A. and Ahmed,A.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (03-SEP-2022) Contact:Amal Adil Kasid Ministry of Higher
            Education and Scientific Research, University of Basra, College of
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Fig. 3. Homo sapiens CXCL10 gene for C-X-C motif chemokine ligand 10

receptors for CCL2, CCL5, or CXCL10 might offer therapeutic potential. Drugs like Plerixafor (CXCR4 antagonist) or Efalizumab (anti-CD11a, involved in T cell trafficking) have been explored, but their impact on these specific chemokines needs further investigation.

Clinical Implications

- Increased Expression of CCL2, CCL5, and CXCL10 in MS Patients: These chemokines may be up-regulated during MS relapse and inflammation, and their levels may correlate with disease severity.
- Therapeutic Targeting: Identifying the role of each chemokine can help develop targeted treatments that modulate the immune response in MS.

Future Recommendations

It might be used in gene expression in MS, genetic variants in MS and chemokine genes and experimental models:

- Tissue Samples: Gene expression levels of CCL2, CCL5, and CXCL10 should be measured in relevant tissues. This can include blood samples (for peripheral immune markers) and cerebrospinal fluid (CSF) or brain tissues (in postmortem MS cases or animal models of MS).
- Real-Time PCR (RT-PCR): One of the most common techniques to quantify gene expression levels. Specific primers for CCL2, CCL5, and CXCL10 can be used to amplify mRNA from samples.
- Western Blotting/ELISA: These methods can be used to measure the protein levels of these chemokines, providing insight into the regulation at the protein level.
- Flow Cytometry: Can be used for analyzing immune cells in circulation and within the CNS that may express chemokine receptors, correlating with the levels of these chemokines.
- Single-Nucleotide Polymorphisms (SNPs): Research has shown that certain genetic variations in the promoters or coding regions of chemokine genes may influence the progression of MS. For example, SNPs in the CCL2 gene could lead to increased expression of the chemokine.
- GWAS (Genome-Wide Association Studies): Genetic studies may reveal associations between variants in CCL2, CCL5, and CXCL10 genes and susceptibility to MS or disease progression.
- Epigenetic Modifications: DNA methylation and histone modification patterns in the promoter regions of these chemokine genes might also play a role in their expression during neuroinflammation.
- EAE (Experimental Autoimmune Encephalomyelitis): This animal model is often used to study MS. Measurement of chemokine expression in EAE models can help understand the role of these chemokines in the development of neuroinflammation and demyelination.
- Humanized MS Models: These models mimic human immune responses and may provide additional insights into how chemokines like CCL2, CCL5, and CXCL10 contribute to MS pathogenesis.

CONCLUSION

New genes for ccl2, ccl5 and cxcl10 chemokines were recorded, and the results are registered in NCBI under accession numbers (LC727557), (LC727558) and (LC727558) respectively. In addition, the understanding the role of CCL2, CCL5, and CXCL10 in neuroinflammation within MS is crucial for identifying potential therapeutic targets.

Their gene expression and associated SNPs in MS patients can contribute to better diagnostics and treatment strategies, potentially slowing disease progression or improving clinical outcomes.

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