Isolation and Characterization of C-C Chemokine Ligand 7 (CCL7) in Cynomolgus Macaques

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ABSTRACT

Cynomolgus macaques (Macaca fascicularis) are an established animal model of asthma, which exhibit different responses to allergen exposure that are clinically relevant. The chemokine ligand gene (CCL7) encodes Monocyte Chemotactic Protein-3, which has an important role in asthma pathogenesis. While CCL7 polymorphism in humans is associated with asthma phenotype, very little is known about CCL7 in nonhuman primate models of respiratory disease. The objective of this study was to isolate and characterize CCL7 gene in cynomolgus macaques of Indonesian origin. In this study, we used sequencing and bioinformatics technique for gene isolation, characterization, and protein 3D structure prediction. We isolated a 2253 base-pair (bp) sequence of CCL7 in cynomolgus macaques, which exhibited 95% similarity in coding sequence to human CCL7. The amino acid sequence was more closely clustered with human CCL7 than with that of rodents. Importantly, the predictive protein structure of CCL7 was similar to that in humans. These similarities in CCL7 suggest the potential of cynomolgus macaque as a translational model to study asthma, particularly in the context of genetics and role of chemokines such as CCL7.

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1. Introduction

Asthma is a chronic disease characterized by inflammation, obstruction, and remodelling of the airways coupled with breathlessness and wheezing (Zafari et al. 2018). Asthma is a worldwide health problem and researchers continuously study genetic factors affecting its pathogenesis and intervention strategies. Nonhuman primates (NHPs) have translational utility in biomedical research due to the high level of genetic homology to humans (Sato and Sasaki 2018), which makes them useful for defining disease mechanisms related to allergic airway disease and COPD in human (Dahlmann and Sewald 2017). Nonetheless, little is known concerning the association of genetic factors with asthma profile in the NHP model. Cynomolgus macaques (Macaca fascicularis) have been used in asthma studies for decades (Cheng et al. 2013; Saul et al. 2014; Nambiar et al. 2015). However, the origin of the species is rarely reported nor considered as a confounding factor despite the potential for genetic variation among Macaca fascicularis of different origins (Shiina et al. 2010). Previous work in our laboratory (unpublished data) showed that Indonesian M. fascicularis exhibited varying responses to allergen challenge to induce deficits in respiratory function and bronchial inflammation, whereby some were non-sensitive to 2017). The expression of CCL7 mRNA increases in the bronchial mucosa of atopic asthma patients (Powell et al. 1996; Lukacs 2001). The association between CCL7 polymorphism and asthma phenotype have been reported in humans (Park et al. 2005; Batra et al. 2011). Importantly, previous study reported that CCL7 expression was high in the airway of asthmatic NHPs (Zou et al. 2002). It is unknown whether polymorphism of this gene also exists in NHPs, specifically in the cynomolgus macaque, due to limited information on CCL7 gene and protein expression in the species.

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Ascaris suum, some required high doses of allergen exposure to elicit asthma response, while others were very sensitive demonstrating immediate allergic asthma profiles following exposure to low doses of allergen. Comprehensive molecular information about CCL7 polymorphism in M. fascicularis is needed to allow further understanding on its relation to disease severity, which may also affect the animal’s use in studies for asthma therapy. Here, Indonesian M. fascicularis CCL7 was characterized by analysis of gene structure, and prediction of 3D protein structure.

2. Materials and Methods

2.1. Ethical Approval

The study was conducted following approval from the Institutional Animal Care and Use Committees of IPB-PRC and PT. Bimana Indomedical, Bogor, Indonesia.

2.2. DNA Isolation and Characterization CCL7

DNA was isolated from Bronchoalveolar Lavage Fluid (BALF) of two male cynomolgus macaques enrolled in an asthma-related study. DNA was amplified and sequenced for cynomolgus CCL7 using eight sets of primers (Park et al. 2005). Sequence analyses were carried out using BioEdit, and multiple alignment using ClustalW. CDS CCL7 sequence used were Homo sapiens (NM_006273.3), Macaca nemestrina (NM_001305906.1), Mus musculus (NM_013654.3), and Rattus novergitus (NM_001007612.1).

2.3. Prediction of 3D Protein Structure

Amino acid sequences were characterized from the exon region data obtained. Prediction of 3D protein structure of CCL7 was carried out using I-TASSER (Zhang 2008). Results were visualized by PyMol.

3. Results

3.1. CCL7 Gene Isolation

We isolated a 2253 bp sequence of M. fascicularis CCL7. Similar to human CCL7, M. fascicularis CCL7 consists of three exons (Figure 1). The Coding Sequence (CDS) of M. fascicularis CCL7 identified in this study was sent to GenBank under accession number MF062250.

3.2. CDS CCL7 Gene Characterization

We isolated 330 bp sequence of M. fascicularis CDS CCL7 from exon region DNA and compared them to those of human, pigtailed macaque and rodents (mouse and rat) (Table 1).

3.3. Prediction of CCL7 3D Protein Structure Using I-TASSER

Figure 2 illustrates the 3D protein structure prediction of M. fascicularis CCL7 analyzed by I-TASSER with the highest C-score of -1.55.

4. Discussion

In this study, we found that M. fascicularis CDS CCL7 consisted of three exons whereas the intron sequence isolated was consistent with the consensus sequence for splice junctions in introns of eukaryotic genes, as it begins with the dinucleotide GU and ends with AG (Uno et al. 2015). The 2028 bp sequence of human CCL7 also consists of three exons, whereby 327–460 bp encodes the functional protein. Human CCL7 mRNA has many UA and AUUUA polyadenylation signal sequences, a

![Figure 1. Exon-intron structures of M. fascicularis and human CCL7 gene. Exon are shown as silver box, intron are shown as black lines](image1)

![Figure 2. Prediction of the 3D protein structure of CCL7. This structure consist of Threonin 40, Threonin 42, Serine 60, and Histidin 68 as receptor binding site (I-TASSER)](image2)

<table>
<thead>
<tr>
<th>CDS vs Amino acid CCL7 (%)</th>
<th>Human</th>
<th>M. fascicularis</th>
<th>M. nemestrina</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>-</td>
<td>92</td>
<td>92</td>
<td>59</td>
<td>62</td>
</tr>
<tr>
<td>M. fascicularis</td>
<td>95</td>
<td>-</td>
<td>98</td>
<td>59</td>
<td>63</td>
</tr>
<tr>
<td>M. nemestrina</td>
<td>96</td>
<td>99</td>
<td>-</td>
<td>59</td>
<td>63</td>
</tr>
<tr>
<td>Mouse</td>
<td>72</td>
<td>72</td>
<td>71</td>
<td>-</td>
<td>88</td>
</tr>
<tr>
<td>Rat</td>
<td>73</td>
<td>72</td>
<td>72</td>
<td>92</td>
<td>-</td>
</tr>
</tbody>
</table>

Lower Δ=% CDS:Upper Δ=% amino acid
characteristic of the chemokine family (Opdenakker et al. 1993). These results indicate similar gene structures of CCL7 in cynomolagus macaques and humans.

Except for human and rodents, the CCL7 sequence of other species are not available in Genbank. The protein-encoding CDS sequence was further analyzed to predict the amino acid sequence and its 3D protein structure. Our results showed *M. fascicularis* CCL7 CDS was 99% and 95% homologous to the CCL7 of *M. nemestrina* and human, respectively. These homology were substantially higher to those of mice and rats (Table 1). This finding was consistent with the amino acid profile showing high similarity with the CCL7 sequence in *M. nemestrina* (98%) and human (92%) compared to rodents (±70%) (Table 1). Our findings are consistent with previous studies on other genes and proteins which reported a high similarity between *M. fascicularis* and humans at the molecular level (Ogawa and Vallender 2014; Uno et al. 2015).

The protein 3D structure of *M. fascicularis* CCL7 was predicted by I-TASSER. This program has been ranked as the best method for prediction of protein structure by critical assessment of protein structure prediction (CASP) experiment (Roy et al. 2010). The predictive protein structure of *M. fascicularis* CCL7 showed the highest C-score of-1.55. C-scores are typically in the range [-5, 2], with a higher score reflecting a higher degree of 3D homology for protein orthologs (Roy et al. 2010). The predicted structure of *M. fascicularis* CCL7 showed 95% homology with human CCL7. I-TASSER results showed that *M. fascicularis* CCL7 is likely to function as a ligand. In humans, CCL7 can bind to three receptors which are CCR1, CCR2, and CCR3 (Lee et al. 2015). The protein 3D structure prediction of *M. fascicularis* CCL7 and its ligand binding are expected to have impacts on the research for studying protein–ligand interaction, protein function and structure-based drug design.

This study support the translational potential of *M. fascicularis* of Indonesian origin as suitable animal model to study mechanisms of inflammation asthma at molecular level, particularly in regards to the role of chemokine such as CCL7.

**Conflict of Interest**

None

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**References**


