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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* suggests 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*.

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.

In humans, C-C Motif Chemokine Ligand plays a key role in asthma pathogenesis. C-C Motif Chemokine Ligand 7 (*CCL7*) is a chemokine that attracts macrophages during inflammation and metastasis (Zhang *et al.* 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.

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

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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

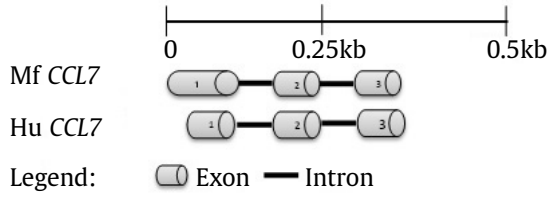

Figure 1. Exon-intron structures of *M. fascicularis* and human *CCL7* gene. Exon are shown as silver box, intron are shown as black lines

Table 1. Comparison of CDS vs amino acid between Human, *M. fascicularis, M. nemestrina,* and rodents

	CDS vs Amino acid *CCL7* (%)				
	Human	*M. fascicularis*	*M. nemestrina*	Mouse	Rat
Human	-	92	92	59	62
M. fascicularis	95	-	98	59	63
M. nemestrina	96	99	-	59	63
Mouse	72	72	71	-	88
Rat	73	72	72	92	-

Lower Δ=% CDS:Upper Δ=% amino acid

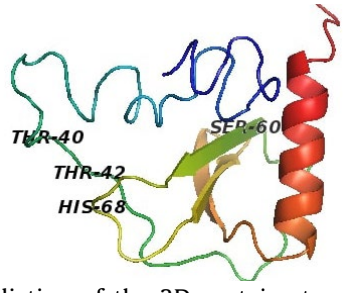

Figure 2. Prediction of the 3D protein structure of *CCL7*. This structure consist of Threonin 40, Threonin 42, Serine 60, and Histidine 68 as receptor binding site (I-TASSER)

characteristic of the chemokine family (Opdenakker *et al.* 1993). These results indicate similar gene structures of *CCL7* in cynomolgus 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 e*t 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 and asthma at molecular level, particularly in regards to the role of chemokine such as *CCL7*.

Conflict of Interest

None

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