CHAPTER 1
INTRODUCTION

1.1 Background

Leber congenital amaurosis (LCA) is one of the most severe retinal dystrophies due to early onset and severe visual impairment. LCA is a genetic disease, mostly autosomal recessive, which caused by mutations in genes involved in retinal development and physiological pathways. Different strategies were used to identify genes involved in this disease, such as linkage analysis, homozygosity mapping and candidate gene analysis combined with next generation sequencing. Up to now, these strategies revealed approximately 70% of LCA cases.\(^\text{1}\)

The molecular genetic defect of LCA can be different among population and ethnicity. Although \textit{CEP290} c.2991+1655A>G mutation is the most common LCA mutation in the Caucasian population, this mutation has not been yet identified in the Indonesian population. Therefore, it is necessary to further investigate about the genetic cause of LCA in Indonesian population, since there are only three paper describing LCA cases.\(^\text{2-4}\) The study of three unrelated families revealed four variants in Indonesian patients. These variants were in \textit{RPE65} gene (106del9bp, G32V, Y435C) and \textit{AIPL1} gene (K14E).\(^\text{2}\)

On the other hand, the advance of gene therapy has been growing rapidly. There are several approaches available: gene augmentation therapy and AON-based therapy. Adeno-associated viruses were used in clinical trials to deliver full-length \textit{RPE65} gene to the retina of LCA patients. This approach proved to be safe and effective, at least up to 3 years follow up.\(^\text{5}\) However, \textit{RPE65} only explained about 3%-16% of LCA cases. One of the most frequent LCA causative gene is \textit{CEP290}. The capacity of adeno-associated virus is 4.7 kb, which is too small to carry full-length of \textit{CEP290} cDNA (~8 kb). Another virus available is lentivirus which has packaging capacity around 8-10 kb. Pre-clinical studies have been done using this vector to carry full-length \textit{CEP290}.\(^\text{7}\) Interestingly, the most common \textit{CEP290}-associated LCA mutation is c.2991+1655A>G, which creates a cryptic splice site in the intronic region, introducing a new exon containing a premature stop codon that way result in a truncated protein. In this case, antisense oligonucleotide (AON) can be used to redirect the splicing pattern, restoring the normal splicing at RNA level.\(^\text{7,8}\)

The use of AONs has several advantages. First, toxic effect caused by overexpression of \textit{CEP290} can be avoided by using AON, which is only working at the pre-mRNA level. Second, AONs are suitable in the recombinant adeno-associated virus (rAAV) construct, which has been shown to be clinically safe and effective for a long-term treatment.\(^\text{5,9}\)
This work consists in the combination of diagnostic and functional studies for LCA disease. The diagnostic study performed to investigate the molecular genetic characteristic of Indonesian LCA patients, while the functional study accomplished to optimize the use of AON in LCA treatment.

1.2 Research Question

1.2.1 What are the molecular genetic causes in Indonesian LCA patients?
1.2.2 Do different AON sequences targeting intronic \textit{CEP290} mutation have different efficiencies in the LCA fibroblast cells at RNA, protein, and cilium levels?
1.2.3 Do different AON chemical modifications show different efficiencies?
1.2.4 What is the lowest effective concentration of AON for LCA fibroblast cells?

1.3 Research Purposes

1.3.1 General Research Purposes
To learn how to identify molecular genetic causes in Indonesian LCA patients and design therapeutic approach for \textit{CEP290}-associated LCA and to learn molecular and cellular techniques

1.3.2 Specific Research Purposes
- To unveil the genetic cause of Indonesian LCA patients
- To establish a genetic counseling for Indonesian LCA cases
- To assess the efficiencies of several AON sequences and chemical modifications.
- To determine the lowest effective concentration of AON.

1.4 Research Benefits

1.4.1 Set a foundation for genetic counseling in LCA and related disease.
1.4.2 Reveal the potential use of AONs for further investigation in treating \textit{CEP290}-associated LCA.

1.5 Research Originality
The patients selected for this study had never been investigated before. Mutation identification strategy used in this study is simple and cost-effective. This is also the functional study that comprehensively encompasses transcriptional, protein, and cilium analysis in order to test 29 different sequences of AON and optimize the potential use of AON in treating LCA.