CHAPTER 7
SUMMARY

Leber congenital amaurosis (LCA) is one of the most severe retinal dystrophies due to early onset and severe visual impairment. 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.[1]

The molecular genetic defect of LCA can be different among population and ethnicity. Although *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 papers describing LCA cases. The study of three unrelated families revealed four variants in Indonesian patients. These variants were in *RPE65* gene (106del9bp, G32V, Y435C) and *AIPL1* gene (K14E).[3]

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 *RPE65* gene to the retina of LCA patients. This approach proved to be safe and effective, at least up to 3 years follow up.[5] However, *RPE65* only explained about 3%-16% of LCA cases.[6] One of the most frequent LCA causative gene is *CEP290*. The capacity of adeno-associated virus is 4.7 kb, which is too small to carry full-length of *CEP290* cDNA (~8 kb). Another virus available is lentivirus which has packaging capacity around 8-10 kb. Lentivirus (LV) is a single-strand RNA retrovirus which is able to infect both dividing and non-dividing cells and can be integrated in the chromosome of host cells. This ability gives a benefit of a long-term expression in dividing cells. However, random insertion of LV in the gene and gene spare long interspersed nuclear elements (LINE) can cause insertional mutagenesis which can lead to other genetic diseases such as cancer. Pre-clinical studies have been done using this vector to carry full-length *CEP290*.[7] Interestingly, the most common *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.[7,8]

The use of AONs has several advantages. First, toxic effect caused by overexpression of *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.[5,9]

This study, which consists of the combination of diagnostic and functional studies for LCA disease, resulted in finding novel variants, p.E318G and p.R324R in *AIPL1* gene. These variants were predicted to recruit splicing factors, thus creating a putative new splice site. Functional studies revealed that AONs sequences predicted by ESE Finder 3.0 program modified with 2'-OMe worked better than new AON sequences that are modified with 2'-MOE. The lowest AON concentration that still efficiently restores correct splicing in LCA patient fibroblast is 0.005µM for GT2, GT3, GT4, and GT22.

Finally, simple technique using several amplicons in this study can be used to screen suspected Indonesian LCA patients in the future. Based on the result of functional studies, GT2, GT3, and GT4 are the most efficient AONs. This study discussed LCA comprehensively, starting from diagnostic study of Indonesian LCA patients to functional study which revealed the potential use of AON as a therapeutic strategy for LCA.