博士論文(要約)

FUNCTIONAL ROLE OF *EFCAB7* AS A CANDIDATE GENETIC MODIFIER OF ELLIS-VAN CREVELD SYNDROME

(Ellis-van Creveld症候群の修飾遺伝子の候補*EFCAB7*の機能解析)

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Ellis-van Creveld syndrome (EVC, MIM ID 225500), a rare ciliopathy, is an autosomal recessive, congenital disorder occurring in about 1 in 60,000 live births. EVC is characterized by disproportionate short stature, polydactyly, dystrophic nails and specific oral defects. More than half of EVC cases have severe congenital heart defects, which lead to early childhood mortality. Causative mutations of either the *EVC* or *EVC2* gene are detected in two-thirds of EVC. *WDR35* mutation has also been reported in some cases. The clinical manifestations of EVC are overlapping with other ciliopathies, which hampers accurate diagnosis. Both the EVC and EVC2 proteins are localized to the basal bodies of the primary cilia and are considered to positively regulate Hh signaling with other molecules. Recently, EFCAB7 and IQCE reportedly co-localized with EVC-EVC2 complex and interact with one another. Based on this finding, I hypothesized that mutations in *EFCAB7* and *IQCE* may also contribute to EVC phenotype. In this study, I conducted clinical and genetic studies of EVC in Vietnam, where no data on EVC have been available so far. Seven unrelated Ellis-van Creveld families within 3 generations were recruited at Children's Hospital 2, Ho Chi Minh City, Vietnam.

All the cases shared common EVC manifestations: moderate to severe short stature, oral abnormalities, nail dystrophy and CHD. Eight of them had polydactyly/syndactyly of hands and feet. Six patients had atrio-ventricular septal defect (AVSD), 2 of them had pulmonary stenosis associated with AVSD. Common atrium, the most severe CHD in EVC, were detected in two cases. Interestingly, two patients in one EVC family had short chordae, an extremely rare CHD, which was never reported in EVC patients.

I screened EVC-complex genes and *WDR35* for the first time in patients with Ellis-van Creveld syndrome in Vietnam. Two compound heterozygous mutations (c.769G>T and c.2476C>T) in *EVC2* were found in patient C2, which was inherited from mother and father, respectively. Both of them were truncated mutations, one was inherited from farther and another from mother. The parents showed variable phenotypes; her father had mild EVC

phenotype while the mother had only short stature. One of the possible explanations for the difference in severity between the parents is somatic mosaic. However, both the parents showed the same mutation in genomic DNA samples from the different tissues. The modulating factors determining the disease severity in each mutation remains unknown.

Compound heterozygous mutations of *EVC* (c.1715C>G-p.S572X, 16.4kb partial deletion-del IVS8-11) and heterozygous *EFCAB7* c.1171T>C (p.Y391H) was detected in one positive-*EVC* family. The genotype-phenotype expression in each family member raised a question whether *EFCAB7* mutation played a role on the pathogenesis of EVC. RNA expression of *EFCAB7* is recognized in various organs, however, protein expression is only detected in fetal heart. It was suggested that EFCAB7 expression is spatiotemporally regulated and important for normal cardiac development. So far, *EFCAB7* mutations have neither been reported as the cause of congenital human diseases, nor as somatic mutations detected in such as neoplasmic tissues. A previous study reported that in vitro depletion of *EFCAB7* downregulates expression of *EVC2* and *IQCE* mRNA. However, the role of missense mutation in *EFCAB7* has not yet been studied. EFCAB7 recruits the EVC-EVC2 complex by interactions between mainly ECH2, a domain of EFCAB7, and the WEYER peptides of EVC2. In this study, a missense mutation located in ECH1 domain, which also binds to WEYER, resulting in a stronger binding to WEYER compared to wild-type ECH1. The complex of WEYER and EFCAB7 activates Hh signaling. The alteration of binding capacity might affect the Hh signaling regulation. Previous studies have observed the binding of EFCAB7 to EVC2, and a notable association of expression levels of EVC-EVC2 and EFCAB7-IQCE. In addition, the presence of interaction partner (EVC to EVC2 and EFCAB7 to IQCE) induces expression of another. Based on these findings, I tested whether mRNA expression of *EVC*, *EVC2* and *IQCE* were altered by transfection of mutated EFCAB7. A possible explanation for the significant lower expression of the binding partner *IQCE* with mutated EFCAB7 than with wild one, was

that the mutated EFCAB7 may be less stable than wild-type EFCAB7. On the contrary, *EVC* mRNA expression was upregulated more in the presence of mutated EFCAB7 compared with wild-type. Recent report revealed that *SUFU* missense variant transfection increased the basal expression levels of the key Sonic Hh signaling - target genes *BCL2*, *GLI1*, and *PTCH1* compared to control. It is suggested that missense variant in a gene could modulate other genes in the same signal transduction pathway. Transfection of shRNA *EVC* together with wild-type *EFCAB7* severely perturbed the expression of *EVC2*. Whereas *EVC2* expression was not altered in mutated *EFCAB7* cells, the *EVC2* mRNA expression level was reassessed (20% of normal level) in co-transfection shRNA *EVC* and *EFCAB7 1171C.* It suggested that mutated *EFCAB7* 1171C might increase *EVC2* mRNA expression in *EVC* knockdown cells. Taken together, both *EFCAB7* and *EVC* may be required for normal *EVC2* transcription. Moreover, the ratio of GLI3R/GLI3FL in co-transfected cells (shRNA *EVC* and mutated *EFCAB7*) was significantly different from that in shRNA *EVC* and wild-type *EFCAB7* also imply that mutated EFCAB7 modulate Hh signaling together with EVC depletion.

In conclusion, I found compound heterozygous mutations of *EVC* and *EVC2* genes in Vietnamese families diagnosed with EVC. The relative expression of *EVC/EVC2* mRNA was reduced in these mutation carriers, which revealed that these mutations were disease-causative. Moreover, *EFCAB7* c.1171T>C (p.Y391H) mutation was firstly co-detected in positive-*EVC* cases, which modified EFCAB7-EVC2 interaction and GLI3 activation in Hh signaling. It suggested that *EFCAB7* is a genetic modifier of EVC.