Invited Lecture

Systematic Clinical, Biochemical, and Molecular Elucidation of Genetic Disorders due to Defective Synthesis of *O*-Mannose Type Sugar Chains, including Fukuyama-type Muscular Dystrophy, and Discovery of Sugar Chains of Novel Structures

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Dr. Tamao Endo graduated from the Faculty of Pharmaceutical Sciences, University of Tokyo in 1977 and obtained his Ph.D. at the same institution in 1982 (under Prof. Shoshichi Nojima). He was a postdoctoral fellow at the Baylor College of Medicine (Prof. Donald M. Marcus), and a research associate at the Institute of Medical Science, University of Tokyo (Prof. Akira Kobata). Since 1994, he has been head of the Department of Glycobiology, Tokyo Metropolitan Institute of Gerontology (TMIG). In 2012, TMIG was reestablished as a new foundation and he was appointed as a vice-director. He served as president of the Japanese Society of Carbohydrate Research from 2011 to 2013 and has served on the editorial board of Glycobiology since 2006. He has received the Tokyo Metropolitan Governor's Award, the Pharmaceutical Society of Japan Award for Divisional Scientific Promotion, the Asahi Award, and the Japan Academy Prize. His current research interests are glycobiology in aging, dementia, and muscular functions.

Dr. Tatsushi Toda is Professor and Chairman of Neurology, Kobe University Graduate School of Medicine. He graduated from the University of Tokyo in 1985 and joined its Department of Neurology. In 1996, he was appointed as an associate professor of the Institute of Medical Science, University of Tokyo. In 2000, he was appointed Professor of Clinical Genetics, Osaka University. He holds his current position since 2009. Dr. Toda has identified genes for Fukuyama muscular dystrophy, muscle-eye-brain disease, and susceptibility to Parkinson's disease. His research interests include the genetics of Parkinson's disease, neuromuscular disorders, higher brain functions, and antisense or sugar therapy for Fukuyama muscular dystrophy. He has received the Japanese Society of Human Genetics Award, the Japan Foundation for Aging and Health Award, the Japanese Society of Neurology Award, the Tokizane Memorial Award, the Asahi Award, Award from the Japanese Minister of Education, Culture, Sports, Science and Technology, and the Japan Academy Prize.

Fukuyama congenital muscular dystrophy (FCMD) is an autosomal-recessive genetic disorder, found almost exclusively in Japan, which is characterized by congenital muscular dystrophy, cobblestone lissencephaly, and eye anomalies. The affected individuals are never able to stand or walk. There was less awareness about this disease, with no known cause or cure. In contrast, studies of glycosylation have a long history, and the significance of glycosylation can be demonstrated by its role in ABO blood group subtype specification. Furthermore, glycosylation is an active field in the progression of various areas of research, including cancer and other disorders, viral infection, and antibody medicine.

Dr. Tatsushi Toda identified the gene responsible for FCMD and laid the foundation for its genetic diagnosis, prenatal diagnosis, and accurate disease-type classification. FCMD is the first human disease known to be caused by an ancient insertion of a "selfish" jumping gene, the SVA retrotransposon, into a functional gene. This SVA insertion occurred in a single ancestor, who lived approximately 2,000 years ago. Upon its discovery, the gene product, fukutin, was a completely new protein of unknown function. In subsequent studies, Dr. Toda discovered that aberrant splicing underlies the molecular pathogenesis of FCMD. SVA insertion activates a rare, alternative donor site in the last exon and creates a new splice-acceptor site in the retrotransposon sequence (exon trapping). This causes incorrect splicing of the mRNA and abnormal "cutting off" of the gene. Assuming that the prevention of this aberrant splicing would lead to the treatment of the disease, Dr. Toda applied an "antisense therapeutic strategy" in which he designed antisense oligonucleotides that bind to the targeted region at the pre-mRNA level to suppress exon trapping. This strategy corrected the abnormal splicing and restored normal fukutin expression in a mouse FCMD model. Dr. Toda aims to conduct clinical trials for future drug approval, thus paving the way for the development of a definitive treatment for FCMD. However, the function of fukutin remained unknown until it was elucidated through collaborative research with Dr. Tamao Endo.

In the late 1990s, Dr. Endo reported that α-dystroglycan, a muscle protein, contains sugar chains linked by mannosyl-serine/threonine groups. This was the first time that the structure of the mammalian *O*-mannosyl glycan was described. A series of enzymatic studies performed by Dr. Endo to elucidate the biosynthetic mechanism of *O*-mannosyl glycans in mammals found that the protein *O*-mannose β1,2-*N*-acetylglucosaminyltransferase (POMGnT1) is involved in the formation of *O*-mannosyl glycan. Dr. Endo and Dr. Toda collaborated and found that muscle-eye-brain disease (MEB), a related disease to FCMD, is inherited as a functional loss of the *POMGnT1* gene. Additionally, a selective deficiency of glycosylated α-dystroglycan was found in MEB patients. These findings indicated that α-dystroglycan is a potential target of POMGnT1, and that hypoglycosylation of α-dystroglycan is the pathological basis of MEB. Therefore, the abnormal muscle and brain phenotypes in MEB can be explained by abnormal glycosylation of α-dystroglycan. This led the researchers to propose a new disease concept, dystroglycanopathy. Dr. Endo also found that the proteins *O*-mannosyltransferase 1 (POMT1) and POMT2 are the initiation enzymes of *O*-mannosyl glycan, and discovered a defect in *POMT1* or *POMT2* genes in Walker–Warburg syndrome (WWS) patients. WWS is another form of congenital muscular dystrophy that is related to MEB. These studies suggested that FCMD may be a glycosylation defect disease because FCMD is related to MEB and WWS.

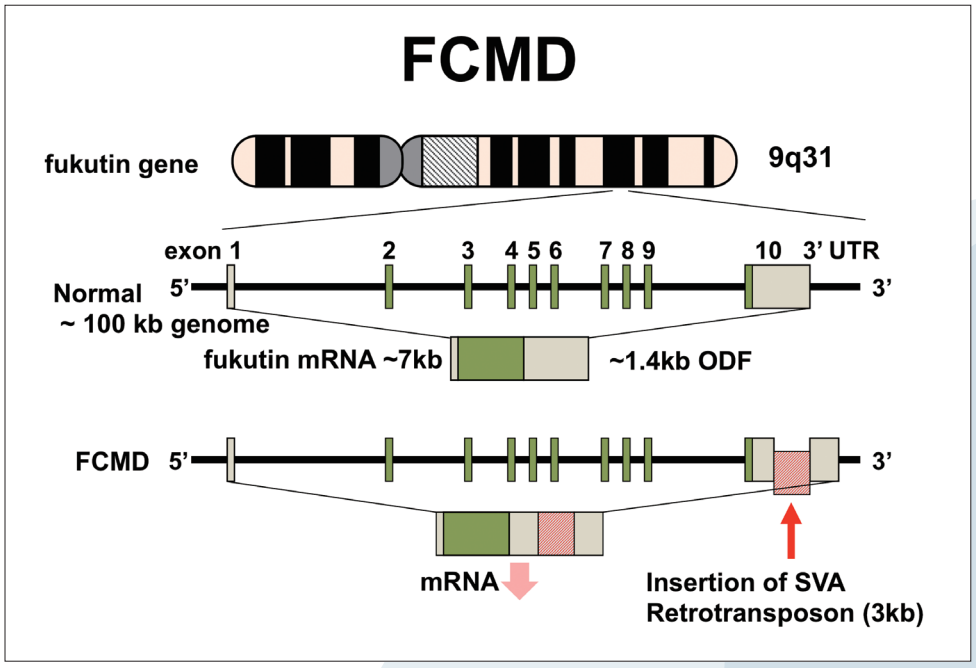

Figure 1
An SVA retrotransposal insertion induces abnormal splicing in FCMD. FCMD is the first human disease known to be caused by an ancient insertion of a "selfish" jumping gene, the SVA retrotransposon, into a functional gene.

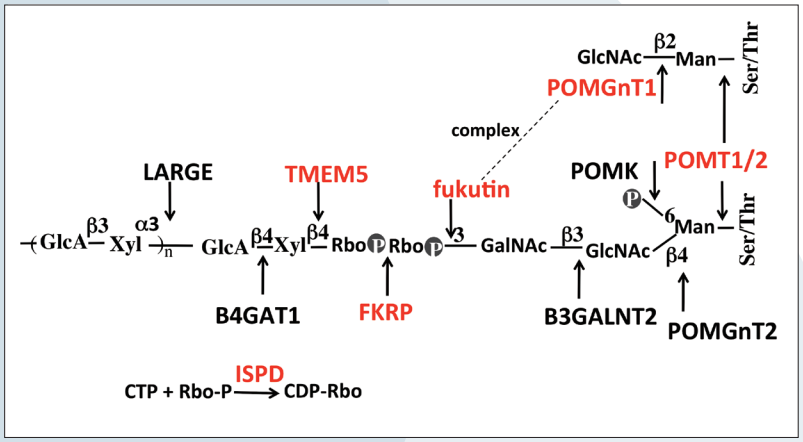

Figure 2
Proposed structure of *O*-mannosyl glycan and its processing glycosyltransferases. The processing enzymes marked in red have been characterized by us. GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; Man, mannose; Rbo, ribitol; Xyl, xylose; B3GALNT2, β1,3-*N*-acetylgalactosaminyltransferase 2; B4GAT1, β1,4-glucuronosyltransferase 1; FKRP, fukutin-related protein; ISPD, isoprenoid synthase domain-containing protein; LARGE, acetylglucosaminyltransferase-like; POMGnT1, protein *O*-linked mannose β-1,2-*N*-acetylglucosaminyltransferase 1; POMGnT2, protein *O*-linked mannose β-1,4-*N*-acetylglucosaminyltransferase 2; POMK, protein *O*-mannose kinase; POMT1/2, protein *O*-mannosyltransferase 1/2; TMEM5, transmembrane protein 5.

To address the functional role of fukutin on the processing of *O*-mannosyl glycan, we continued to collaborate to elucidate the *O*-mannosyl glycan structure. Recently, we proposed the entire structure of *O*-mannosyl glycan, [3GlcAβ1,3Xylα1,]$_n$ 3GlcAβ1,4Xylβ1, 4Rbo5P,1Rbo5P,3GalNAcβ1,3GlcNAcβ1,4(phosphate-6)Man, which is required for the binding of α-dystroglycan to extracellular matrix proteins. The tandem Rbo5P, a phosphoric ester of pentitol, is unique. We determined three enzymes to be involved in the synthesis of tandem Rbo5P: isoprenoid synthase domain-containing protein (ISPD), a CDP-Rbo synthetase, and fukutin and fukutin-related protein (FKRP), Rbo5P transferases that utilize CDP-Rbo.

POMGnT1 is causative for MEB, as described above. Although POMGnT1 does not catalyze any reactions involved in the above-proposed *O*-mannosyl glycan structure, *POMGnT1* KO mice and MEB patients lack the GlcA-Xyl repeat. This suggests that POMGnT1 plays an important role in the processing of GalNAcβ1,3GlcNAcβ1, 4(phosphate-6)Man. Fukutin forms a complex with POMGnT1, and the POMGnT1 crystal structure reveals that the stem domain of POMGnT1 recognizes GalNAcβ1,3GlcNAc. Since fukutin is required for the first Rbo5P modification of the GalNAcβ1,3GlcNAcβ1,4(phosphate-6)Man glycan, the POMGnT1-fukutin complex is important in forming a platform that requires further glycosylation. These findings expand our knowledge on glycosylation machinery.

Our joint research between genomic medicine/clinical genetics and carbohydrate biochemistry has established that the elusive cause of FCMD and other related muscular dystrophies is the defective synthesis of *O*-mannose sugar chains on α-dystroglycan. Furthermore, the discovery of a new sugar chain and its significance further contributed to the recognition of sugar chains as an important biological material. We anticipate that our findings will lead to new treatments for these diseases.

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