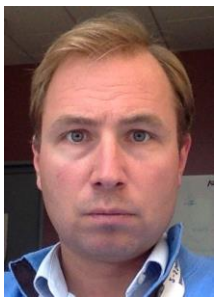
Principal Investigator: Dr François FOULQUIER

Grant Title: TMEM165, a new key player in Golgi glycosylation and skeletal development.

Abstract:



Congenital Disorders of Glycosylation (CDG) are a rapidly growing disease family due to genetic defects of protein and lipid glycosylation. In 2012, we reported a novel disorder in this group namely TMEM165-CDG (OMIM entry #614727). These patients present a peculiar phenotype including major skeletal dysplasia and hyposialylation and hypogalactosylation of N-glycosylproteins. TMEM165 is a transmembrane protein of 324 amino acids belonging to a well conserved but uncharacterized family of membrane proteins named UPF0016 (Uncharacterized Protein Family 0016; Pfam PF01169). Extremely conserved in the eukaryotic reign, we wanted to investigate the molecular mechanisms by which TMEM165 deficiencies lead to Golgi glycosylation abnormalities is enigmatic.

As GDT1 is the ortholog of TMEM165 in yeast, both gdt1Δ null mutant yeasts and TMEM165 depleted cells were used. Strong galactosylation defects on N-glycoproteins were observed in TMEM165 deficient cells. We highlighted that the observed Golgi glycosylation defects due to Gdt1p/TMEM165 deficiency result in fact from Golgi manganese homeostasis defect (figure 1). We discovered that in both yeasts and mammalian Gdt1p/TMEM165 deficient cells, Mn2+ supplementation could restore a normal glycosylation. We also showed that the GPP130 Mn2+ sensitivity was altered in TMEM165 depleted cells. To go deeper in the molecular mechanisms, we investigated the sensitivity of TMEM165 for Mn2+. When exposed to high Mn2+ concentrations, we demonstrated that TMEM165 was degraded into lysosomes. Remarkably, the variant p.E108G recently identified in a novel TMEM165-CDG patient, was found to be insensitive to Mn2+ supplementation. Moreover, this mutation abolished the function of TMEM165, suggesting that a transport function may be necessary for its regulation. Altogether this grant allowed us to identify the Golgi protein TMEM165 as a novel cytosolic Mn2+ sensor in mammalian cells and pointed the crucial importance of the cytosolical ELGDK motif in both Mn2+ sensitivity and function. Moreover, we demonstrated that the observed Golgi glycosylation deficiencies in Gdt1p/TMEM165 deficient cells result from a defective Golgi Mn2+ homeostasis then providing novel insights into the mechanism of the galactosylation defect observed in TMEM165-deficient cells. These findings also support the potential use of therapeutic trials of Mn2+ in TMEM165 deficient patients.

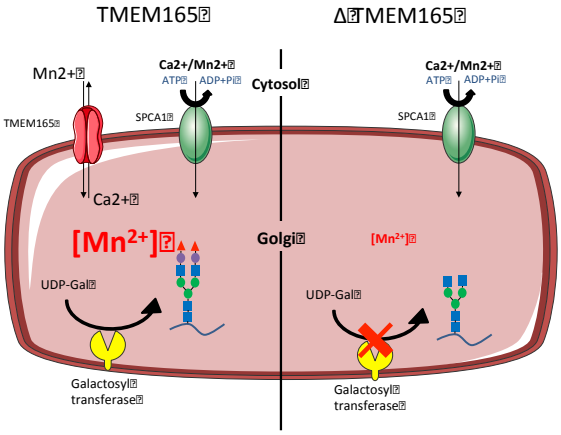


Figure 1: Schematic representation of the impact of TMEM165 deficiency on Golgi Mn^{2+} homeostasis and N-glycosylation defects.

1_ Potelle S, Morelle W, Dulary E, Duvet S, Vicogne D, Spriet C, Krzewinski-Recchi MA, Morsomme P, Jaeken J, Matthijs G, De Bettignies G, Foulquier F. (2016) Glycosylation abnormalities in Gdt1p/TMEM165 deficient cells result from a defect in Golgi manganese homeostasis. ***Hum Mol Genet***. 25, 1489-500.

2_ Potelle S, Dulary E, Climer L, Duvet S, Morelle W, Vicogne D, Spriet C, Krzewinski-Recchi MA, Peanne R, De Bettignies G, Matthijs G, Marquardt T, Lupashin V, Foulquier F. (2016) Characterization of TMEM165 as a novel Golgi manganese sensitive protein involved in Congenital Disorders of Glycosylation. ***Submitted to Hum Mol Genet***.