Genetic Ablation of TMEM16F Exhibits Strain-specific Lethality in Mice

Colin A Kretz, Univ of Michigan, Ann Arbor, MI; Gary Gilbert, Brigham and Women's Hosp, Boston, MA; David Ginsburg, Univ of Michigan, Ann Arbor, MI

Background: Scott Syndrome is a rare bleeding disorder characterized by a defect in platelet phosphatidylserine (PS) exposure. The syndrome has recently been linked to mutations in TMEM16F. *Tmem16f*$^{-/-}$ mice were recently reported to be viable with a prolonged tail snip bleeding time but no spontaneous bleeding. We now report analysis of an additional gene targeted *Tmem16f* allele generated in C57BL/6 ES cells.

Results: JM8 ES cell were obtained from EUCOMM, and successful Tmem16f gene targeting in intron 1 was confirmed by PCR and sequencing. Genotyping of 120 *Tmem16f*$^{+/gt}$ (+/gt) intercross progeny identified no surviving *Tmem16f*$^{gt/gt}$ (gt/gt) mice at weaning ($p<0.001$). However, +/gt intercrosses generated the expected Mendelian genotype ratios at both E10.5 and E17.5, with gt/gt embryo's exhibiting no morphological abnormalities on gross or routine histologic examination. Though complete deficiency of TMEM16F is lethal in the C57BL/6J genetic background between E17.5 and birth, an F2 intercross of +/gt mice outcrossed one generation to 129x1SvJ resulted in gt/gt mice surviving to weaning, though at reduced numbers (6/75 total progeny compared to ~19 expected, $p <0.002$). Progeny testing of surviving gt/gt mice suggest a single autosomal dominant 129x1SvJ-associated genetic modifier. Preliminary genetic analysis of these mice appears to map this locus to the proximal region of chromosome 3.
Tail bleeding times for gt/gt were >10min, whereas littermate +/gt and +/+ mice bleeding ceased at 8 ± 1 min and 6 ± 0.8 min, respectively, each significantly different than gt/gt ($p<0.05$). Notably, platelets from +/gt mice exhibited a trend toward reduced PS exposure, detected with FITC-labelled lactadherin, in response to PAR4 agonist peptide, whereas gt/gt mice had significantly reduced PS exposure ($p < 0.05$).

Conclusion: These data suggest the existence of a viability-determining genetic modifier of TMEM16F in the 129x1SvJ mouse strain. Identification of the responsible gene may uncover novel functions for TMEM16F and the regulation of hemostatic function.

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