Characterising loss of transcription factor Gfi1b in megakaryocytes in murine bone marrow and spleen tissues by transmission electron microscopy

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Background: GFI1B is a transcription factor that is important for normal red blood cell and megakaryocyte/platelet differentiation and development. A bleeding disorder, caused by a single nucleotide insertion into the *GFI1B* gene (c.880_881insC), with platelet changes consistent with aberrant platelet and red blood cell formation has been found in a four-generation family (Stevenson et al, 2013). Investigations into this family have included the establishment of a mouse model to characterise loss of gene function of murine *Gfi1b* in the megakaryocyte lineage.

Aim: To further characterise the role of the *Gfi1b* gene on megakaryocyte /platelet development, a morphology study on bone marrow and spleen from *Gfi1b* conditional knockout (KO) mice and control mice was carried out. Transmission electron microscopy (TEM) was used to compare features of megakaryocytes from these tissues.

Methods: Bone marrow and spleen tissue from mice with *Gfi1b* conditionally knocked out in the megakaryocyte lineage (platelet factor 4 (PF4)-Cre$^{T/+}$ x floxed *Gfi1b, Gfi1b*$^{fl/fl}$*)* (Tiedt et al, 2007; KOMP Repository) and from control mice with intact Gfi1b$^{fl/fl}$*,* were harvested, fixed in glutaraldehyde and processed for routine TEM.

Results: 38 megakaryocytes in total from the bone marrow and spleens of 2 control and 2 *Gfi1b* conditional KO mice were analysed. Control mice tissue showed prominent alpha granules (**g**), structured demarcation membrane systems (**DMS**) extending around the nucleus, and well-defined platelet territories (**pt**) in the cytoplasm of the megakaryocytes in both bone marrow and spleen (**Figure 1**). In contrast, KO mice tissue displayed no alpha granules, poorly defined or no platelet territories, and immature DMS, concentrated at one end of the megakaryocyte, in both types of tissue. Evidence of normal platelet formation in the *Gfi1b* conditional knockout mice was not seen.

Conclusions: The conditional knockout of the *Gfi1b* gene along the megakaryocyte lineage prevented the normal development of platelets from megakaryocytes. The use of transmission electron microscopy provided an important insight into the intracellular changes during the maturation process of megakaryocytes, and allowed direct comparison of these changes between control and *Gfi1b* KO mice in the investigation of this bleeding disorder.

References:

KOMP Repository (www.komp.org)

Stevenson WS, Morel-Kopp MC, Chen Q, Liang HP, Bromhead CJ, Wright S, et al. GFI1B mutation causes a bleeding disorder with abnormal platelet function. *J Thromb Haemost*. 2013;11(11):2039-47. doi: 10.1111/jth.12368.

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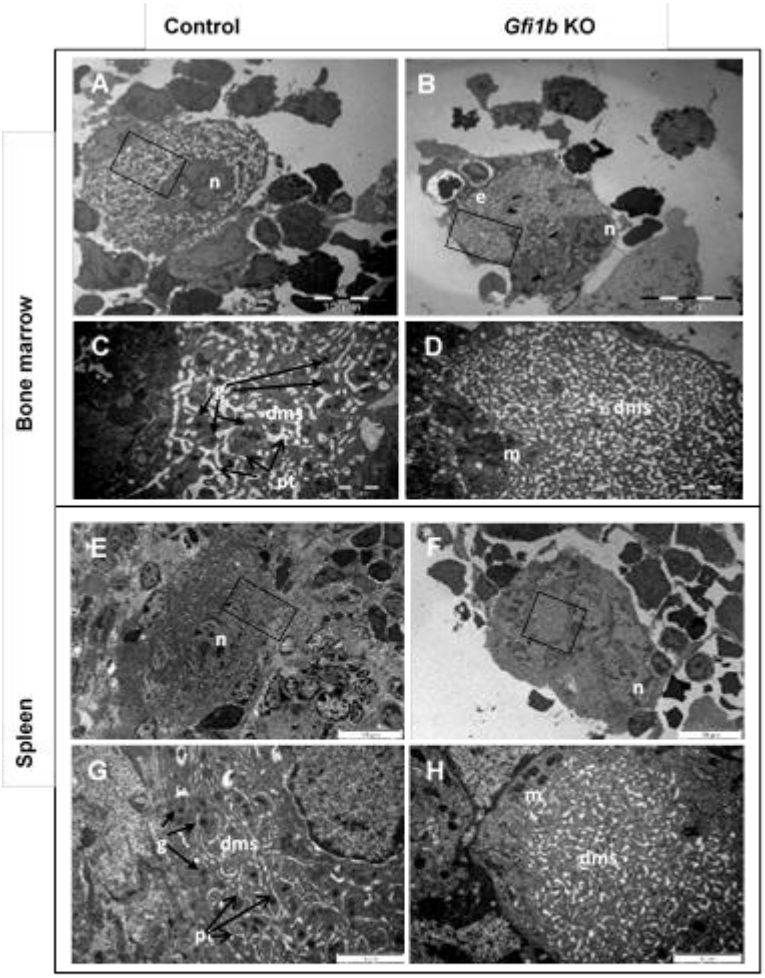

Figure 1. Transmission electron micrographs of representative megakaryocytes in bone marrow and in spleen from control mice and *Gfi1b* conditional knockout mice. (A), (B), (E), (F): Magnification x 2500. (C), (D), (G), (H): Magnification of boxed regions from (A), (B), (E), and (F) respectively (magnification x 13500).
e, emperipolesis (2 cells contained within the cytoplasm); **n**, nucleus; **dms**, demarcation membrane system; **pt**, platelet territories; **g**, alpha granules, **m**, mitochondria.