Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602102650

Quantitative Analysis of Cerebral Corrosion Casts from *Endoglin* $^{+/+}$ and $^{+/-}$ Mice

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Hereditary hemorrhagic telangiectasia (HHT) is a genetic vascular disorder characterized by dilated vessels and arteriovenous malformations resulting from mutations in the genes coding for *Endoglin* (*Eng*), (HHT1) or *ALK-1* (HHT2). Two inbred strains of mice, C57BL/6 (B6) and 129/Ola (129), each expressing one *Eng* allele were generated as an HHT1 model [1, 2]. The aim of the present study is to use vascular corrosion cast techniques to identify morphological differences in cerebral vasculature between $Eng^{+/+}$ and $Eng^{+/-}$ mice and strain differences between B6 and 129. Twenty five mice (5/group) aged 25-40 weeks were examined: wild type B6 (B6WT); B6 $Eng^{+/+}$ generations N5 or N6; B6 $Eng^{+/-}$ N5 or N6; 129 $Eng^{+/+}$; and 129 $Eng^{+/-}$.

Corrosion casts were prepared by the following procedure: anesthetize with intraperitoneal Nembutal (50mg/kg), perfuse the ascending aorta with 20 ml heparinized PBS (10,000U/500ml), inject 10 ml resin (Batson #17) at 1ml/min, polymerize at RT for 24 h, remove the brain and macerate in 20% KOH at RT for 24-72 h with intermittent distilled water rinses, air dry, sputter-coat with gold, examine by scanning electron microscopy. For quantitative analysis the following parameters were determined: arterial diameter; constriction and dilatation in pre-capillary arterioles; endothelial nuclei pattern (length, width, area and orientation). Measurements were taken from micrographs using "The Imaging Processing Tool Kit" under Adobe Photoshop, the operator was blinded to specimen identification. Groups were compared using Generalised Estimation Equations to allow analysis of repeated measures from each animal.

There was no significant effect of age or sex. To identify any gross variation in the vasculature of the 5 groups the diameters of the proximal middle cerebral artery and the parent artery of selected pre-capillary arterioles (line 1, fig. 1) were measured; no significant differences were found between or within groups. Constriction and dilatation were measured in 10 pre-capillary arterioles/mouse (n=50/group). Pre-capillary constriction, sphincters regulating blood flow to the capillaries, was defined as origin/distal diameters <1 (lines 2 & 4, fig. 1). Constriction was significantly less prevalent in both $Eng^{+/-}$ groups when compared to their $Eng^{+/+}$ counterparts (B6, $P<0.001$; 129, $P<0.05$). B6WT had significantly more constrictions than the 129 strain ($P<0.001$) but was not different from B6 $Eng^{+/+}$ ($P=0.38$). Dilatation, vessel swelling associated with HHT, was defined as proximal/distal diameters >1 (lines 3 & 4, fig. 1). Dilatation was significantly greater in B6 $Eng^{+/-}$ than in the $Eng^{+/+}$ variant ($P<0.05$); no significant difference was found between the other groups.

The structure of the vascular wall luminal surface was quantified by measuring the impressions of 50 endothelial nuclei from a minimum of 5 pre-capillary arterioles per mouse (n=250/group) (fig. 2). Nuclei located at the edge of an arterial image or near a branching point were excluded. Nuclei length (maximum distance between two points), width (maximum distance of a line perpendicular to length) and area were determined. B6 $Eng^{+/-}$ nuclei were significantly smaller in area than the $Eng^{+/+}$ variant ($P<0.01$); no significant difference was found between other groups. $Eng^{+/-}$ groups

were significantly rounder (width/length) than *Eng*$^{+/+}$ (B6, P<0.005; 129, P<0.0001); no strain differences were noted. Cast images were aligned to assess nuclei orientation relative to direction of blood flow. Deviation from the direction of blood flow was greater in both *Eng*$^{+/-}$ groups relative to the *Eng*$^{+/+}$ groups (B6, P<0.05; 129, P<0.0001);); no strain differences were noted.

Eng$^{+/+}$ and *Eng*$^{+/-}$ mice can be differentiated by analysis of cerebral pre-capillary arteriole corrosion casts. The *Eng*$^{+/-}$ variant has less branching point constrictions, more arteriole dilatations, and rounder and less organized endothelial nuclei. 129 has a reduction in branching point constrictions compared to B6; no other significant strain differences were noted.

References

[1] A. Bourdeau et al., *Trends Cardivasc Med.* 10 (2000) 279.
[2] A. Bourdeau et al., *Am J Pathol.* 158 (2001) 2011.

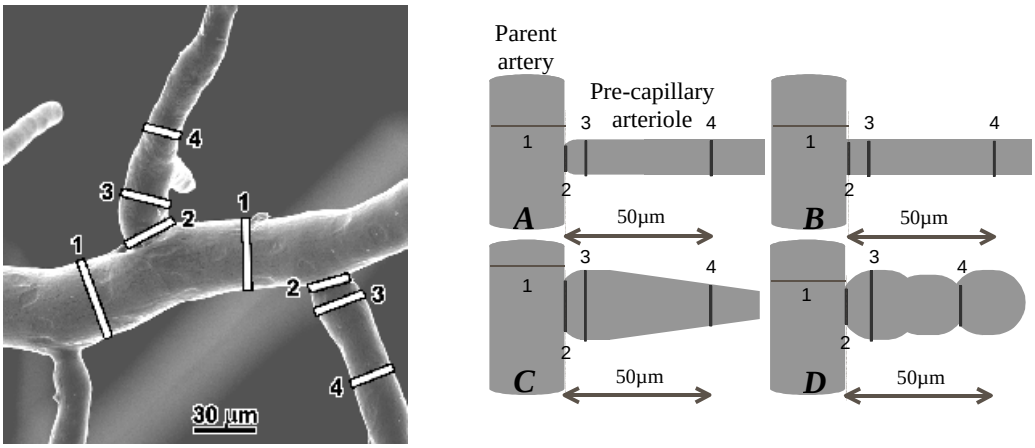

FIG. 1. Left: Measurements taken from corrosion casts of cerebral vasculature. Diameter at: 1, parent artery; 2, point of origin; 3, proximal; 4, distal. Right: Location of measurements in cases of; A) constriction, B) no constriction, C) proximal dilatation, D) proximal and distal dilatation.

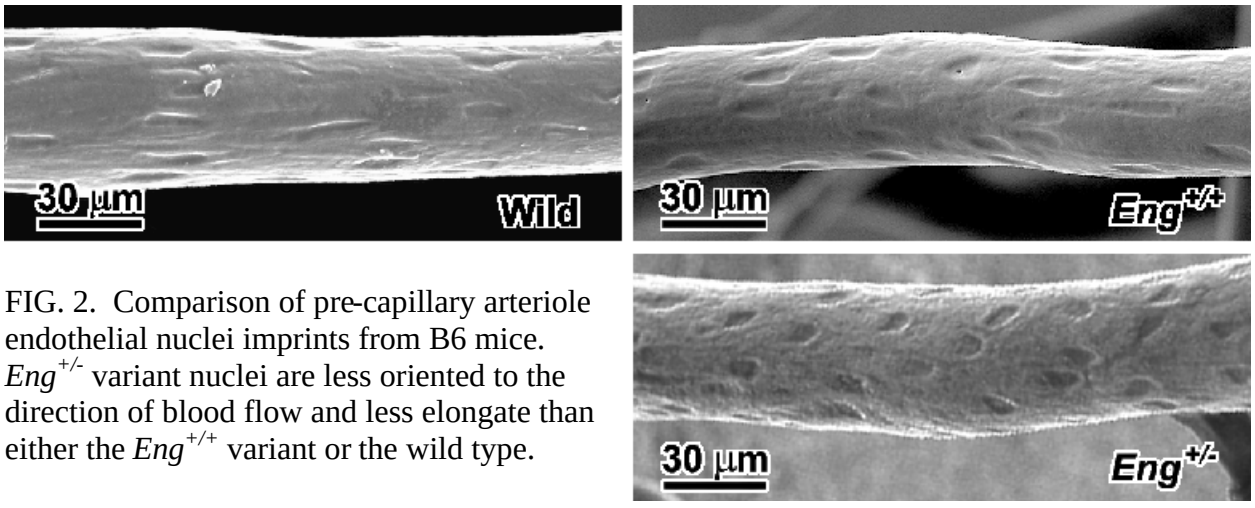

FIG. 2. Comparison of pre-capillary arteriole endothelial nuclei imprints from B6 mice. *Eng*$^{+/-}$ variant nuclei are less oriented to the direction of blood flow and less elongate than either the *Eng*$^{+/+}$ variant or the wild type.

https://doi.org/10.1017/S1431927602102650 Published online by Cambridge University Press