Phenotype analysis of Zfp516 -/- and Zfp516 -/+ mouse embryos based on High resolution episcopic microscopy

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Objective

In mice *Zfp516* is known to regulate brown fat tissue formation and stemness in embryonic stem cells (Dempersmier et al., 2015; Sambeat et al., 2016; Kwak et al., 2018). Loss of its function leads to embryonic lethality. Yet, information whether morphogenesis and fetal anatomy is abnormal in homozygous and heterozygous mice is scarce and contradicting. The function of the gene is therefore still not fully characterised and it is not yet clear whether it fits as model for studying the function of the human orthologue *ZNF516*. Our study aims at providing comprehensive and high detail information on the morphological phenotype of homozygous and heterozygous harvested at embryonic day (E) 14.5.

Material and Methods

High resolution episcopic microscopy (HREM) was used to create digital volume data with voxel sizes of 3x3x3 µm³ from 4 *Zfp516-/-* and 10 *Zfp516-/+* E14.5 mouse embryos. A standardised protocol, involving scrolling through the images of all three body planes, virtual slicing, volume and surface rendering was followed to perform systematic phenotype analysis (Weninger et al., 2014).

Results

Both, Zfp-/- and Zfp-/+ E14.5 embryos showed a wide range of structural abnormalities, in particular of the cardiovascular and nervous systems. Four homozygous and two heterozygous embryos showed perimembraneous or muscular ventricular septal defects. Four homozygous and two heterozygous embryos showed brain defects or cranial nerve abnormalities.

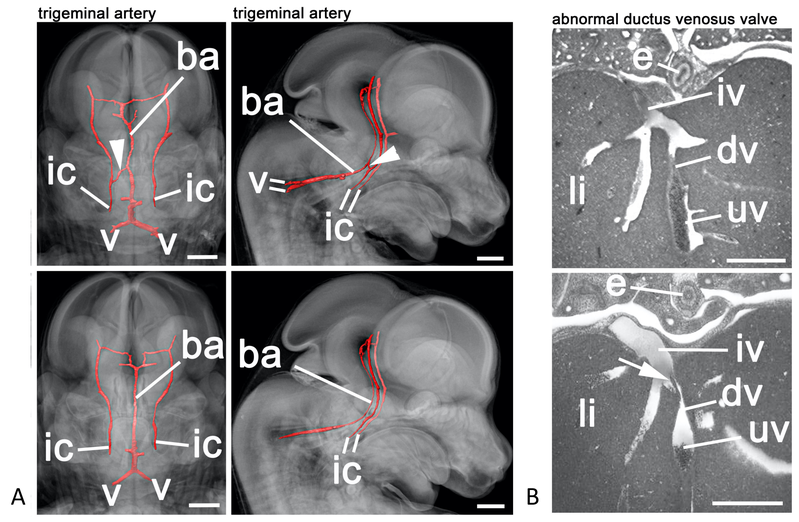

Figure 2: Vascular abnormalities. (A) Persistent trigeminal artery visible in a surface rendered model of large head arteries integrated in semitransparent volume rendered models of the head and neck from ventral (left) and lateral (right). The trigeminal artery, indicated by the white arrowhead, is connecting the right internal carotid (ic) and basilar (ba) arteries. (B) Absent ductus venosus valve (indicated in control with white arrow). dv, ductus venosus; e, esophagus; iv, inferior vena cava; uv, umbilical vein; v, vertebral artery. Scale bars: 500 µm.

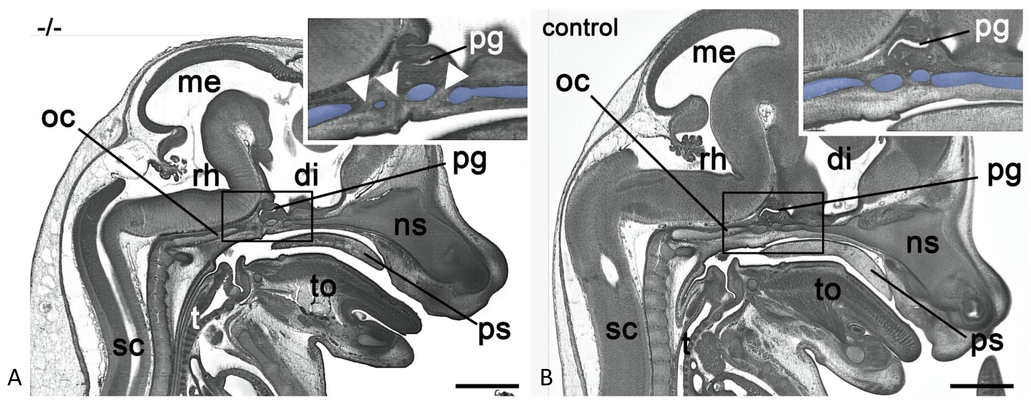

Figure 3: (A) Abnormally sized foramina (arrowheads) in midline of skull base (blue overlay in inlay). Sagittal re-section through the head. Ventral to the right. (B) Control. di, diencephalon; me, mesencephalon; ns, nasal septum; oc, occipital bone; pg, pituitary gland; ps, palatine shelf; rh, rhombencephalon; sc, spinal cord; t, trachea; to, tongue. Scale bars 1 mm.

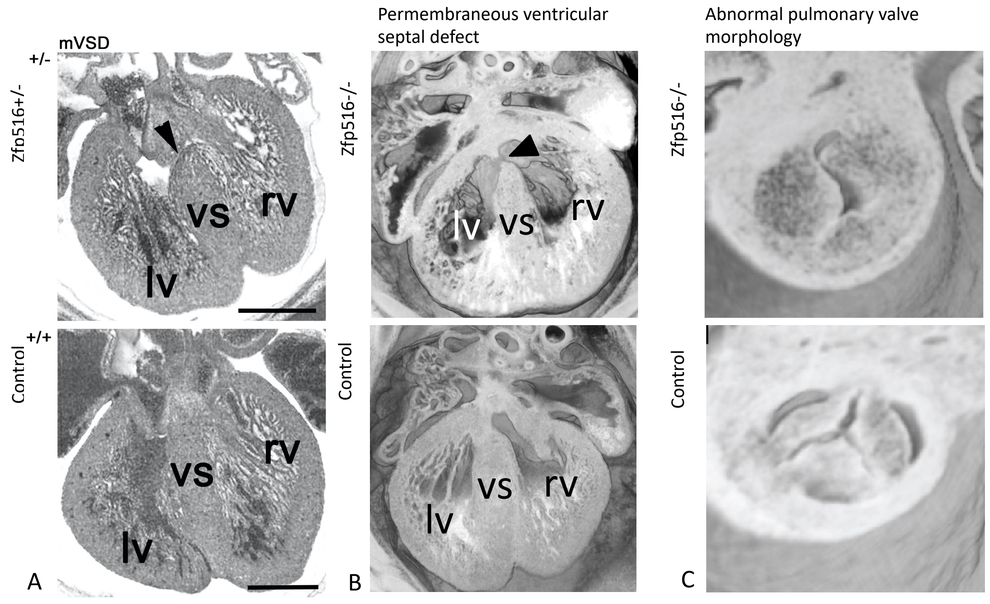

Figure 1: Cardiac defects. (A) Small muscular ventricular septal defect (mVSD) (arrowhead) in a ZFP516+/- embryo. Axial section through the heart from cranial. (B) Perimembraneous ventricular septal defect (pVSD) (arrowhead) in a Zfp516-/- embryo. Axial section through a volume rendered model from cranial. (C) Abnormal pulmonary valve morphology in a Zfp516-/- embryo, volume rendering. lv, left ventricle; rv, right ventricle; vs, ventricular septum. Scale bar: 500 µm.

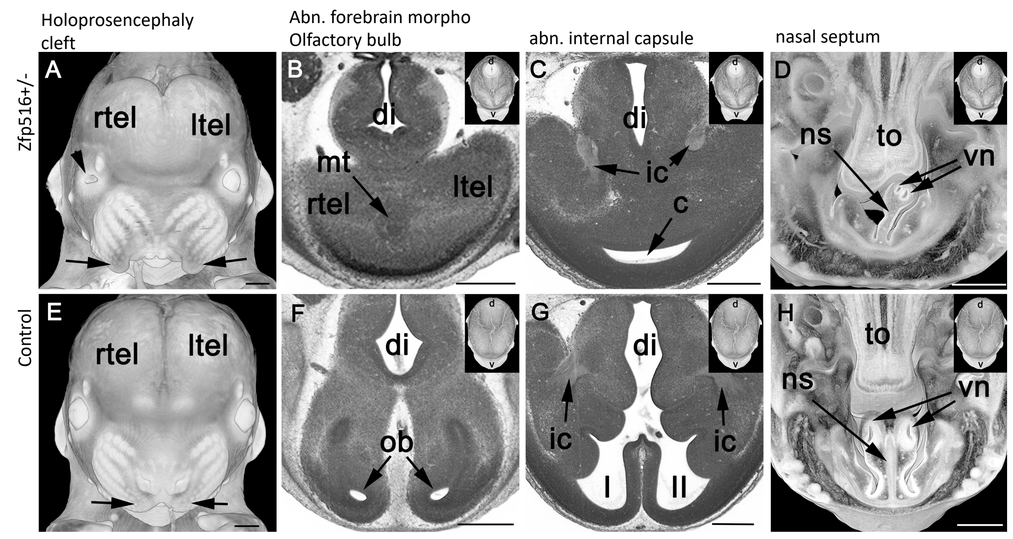

Figure 4: Cranial malformations in E14.5 Zpf heterozygotes. (A-C) Holoprosencephaly in a +/- embryo with rostrally fused left (ltel) and right (rtel) telencephalic hemispheres, connected (c) left (I) and right (II) ventricles and missing olfactory bulbs. Note the slit like malformation of the eye opening (arrowhead) and the gap between left and right maxilla and lip (arrow) in A, the abnormal brain tissue (mt) in the midline in B and the abnormal internal capsule (ic) in C. (D) Abnormal nasal septum (ns) in a +/- embryo. Note the deviation and missing cartilage primordium of the osseous septum. (E-H) Comparable images of controls matching in developmental stage. d, dorsal; di, diencephalon; to, tongue; v, ventral; vn, vomeronasal organ. Scalebars 500 µm.

Conclusion

Our analysis demonstrate that Zfp516 plays an essential role in cardiovascular formation and the development of the nervous system.

Zfp516 plays role in development of various organ systems

- Fully penetrant heart defects
- Essential role for nervous system
- Urogenital system

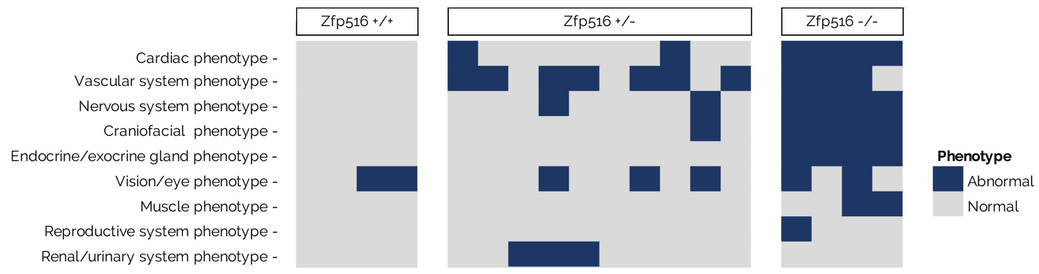

Figure 5: Phenotypes observed in Zfp516 knock out and WT embryos. The distinct phenotypes identified through whole-body HREM analysis for each embryo. Phenotypes were mapped onto the broad set of ontology categories defined by the programme Deciphering the Mechanisms of Developmental Disorders (DMDD).

References

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