# Targeting of podocyte specific genes in zebrafish *(Danio rerio)* using morpholinos

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Glomerular epithelial cells (podocytes) play a key role in the development of progressive glomerulosclerosis. They are considered incapable of replication postnatally and the ability of the glomerulus to compensate for podocyte loss may be insufficient. Recently, genetic approaches to identify disease genes for various familial nephrosis and focal-segmental glomerulosclerosis (FSGS) syndromes have led to the discovery of defects in podocyte proteins (nephrin, α-actinin-4, podocin, cd2ap) that are functionally related at the molecular interphase linking integral cell surface proteins to the actin cytoskeleton1. The zebrafish (Danio rerio) is a very good model-species to examine the direct effects of gene targeting in a vertebrate. This is particular the case in the context of kidney development, since the zebrafish development occurs rapidly from the fertilized egg to free-swimming larvae in 2.5 days. Since zebrafish embryos are transparent internal organ development can be observed even without dissection. The functional kidney in the zebrafish is the pronephros, a very simplified organ structure with only two nephrons with glomeruli fused at the midline that contain the full range of cell types typical of kidneys of higher vertebrates. The glomerular filter also contains a fenestrated endothelial cell layer, a trilaminar glomerular basement membrane (GBM) and podocytes with elaborate foot processes2. The aim of our studies was to establish an in vivo system to examine genetic ablation of relevant genes for podocyte function in a high throughput assay system. We specifically targeted genes in zebrafish that are known to lead to a podocyte related renal phenotype in mammals and disrupted their expression using morpholino techniques. The morpholinos (2.5ng-7ng ) were microinjected in fertilized zebrafish eggs with an average injection volume of 0.3nl using a Nanoject II (Drummond). Kidney development and phenotype changes were examined 24, 48, 72 and 96 hours after fertilization. We used morpholinos for the predicted zebrafish orthologues for podocin3 (a component of the slit diaphragm mutated in autosomal recessive steroid-resistant nephrotic syndrome), CD2AP4 (an adaptor molecule anchoring podocin to the slit-diaphragm and involved in intracellular survival signaling) and TRPC65 (a non selective cation-channel mutated in a familial form of FSGS). Injection of these morpholinos induced a fetal hydrops in zebrafish larvae with pericardial effusion, internal hydrocephalus and yolk sac edema compared to no effects observed in zebrafish larvae injected with control (scrambled) morpholinos (figure 1). Functional studies revealed leakage of high molecular weight proteins into the urine, demonstrating proteinuria in morpholino injected zebrafish embryos.

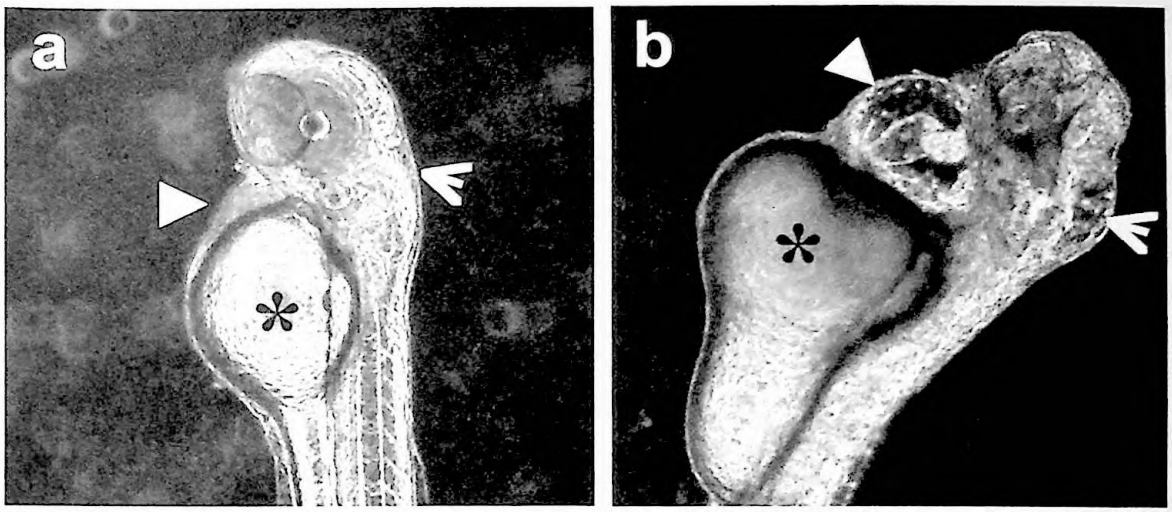


Figure 1: Phenotype changes in living zebrafish after injection of (a) control morpholino or (b) podocin-morpholino. White full arrow indicates pericadial effusion, white open arrow indicates development of hydrocephalus and asterix demonstrates yolk sac edema 96 hours post fertilization in podocin-morpholino injected zebrafish larvae.

Ultrastructurally we could demonstrate failure to form normal foot processes. These data support our hypothesis that the developing zebrafish is a suitable high-thoughput assay system to examine the potential relevance of unknown genes for the normal barrier function of podocytes.

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