Dual function of the retinoic acid catabolizing enzyme CYP26C1:
(I) modifying disease severity in SHOX deficiency and
(II) underlying idiopathic short stature

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To elucidate the factors that modify disease severity/penetrance in short stature, we have studied a three-generation family with SHOX deficiency. We have found that the retinoic acid degrading enzyme CYP26C1 is a modifier for SHOX deficiency phenotypes towards the more severe clinical manifestations (Leri-Weill dyschondrosteosis) and confirmed these findings in independent cases. We also asked whether damaging variants in *CYP26C1* alone could lead to short stature. We performed exome and Sanger sequencing to analyze 856 individuals with short stature where SHOX deficiency was previously excluded. Three different damaging missense variants and one splicing variant were identified in six independent individuals. The functional significance of the identified variants was tested *in vitro* (splicing defect) or *in vivo* (missense mutations) using Zebrafish as a model. The identified *CYP26C1* variants affected the catabolic activity of *CYP26C1* in human primary chondrocytes and zebrafish embryos. Together, the genetic and functional data reported here indicate that *CYP26C1* represents a novel gene underlying growth disorders with dual function: damaging variants in *CYP26C1* in the absence of *SHOX* mutations can lead to short stature and damaging variants in *CYP26C1* modify SHOX deficiency phenotypic outcomes through the retinoic acid signaling pathway.

I. Modifier in Leri-Weill Dyschondrosteosis

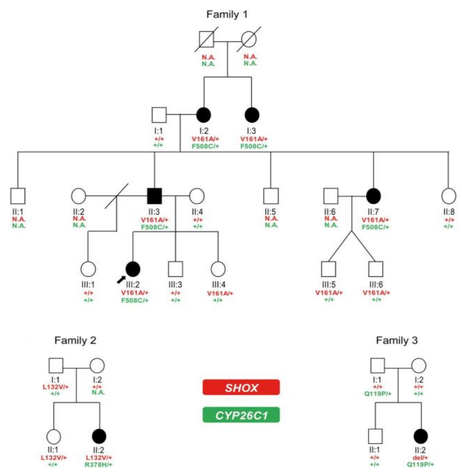

Figure 1. Identification of *CYP26C1* as a genetic modifier
Damaging variants in SHOX and *CYP26C1* in patients with LWD.

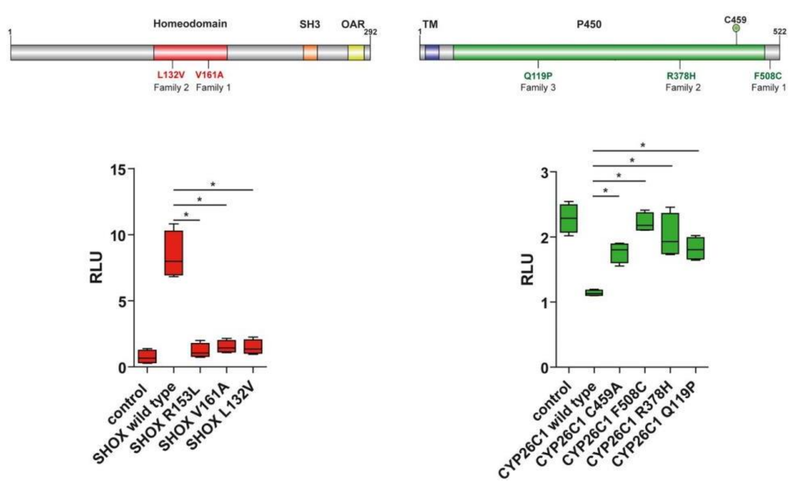

Figure 2. Scheme of *SHOX* and *CYP26C1* and results of Luciferase assays

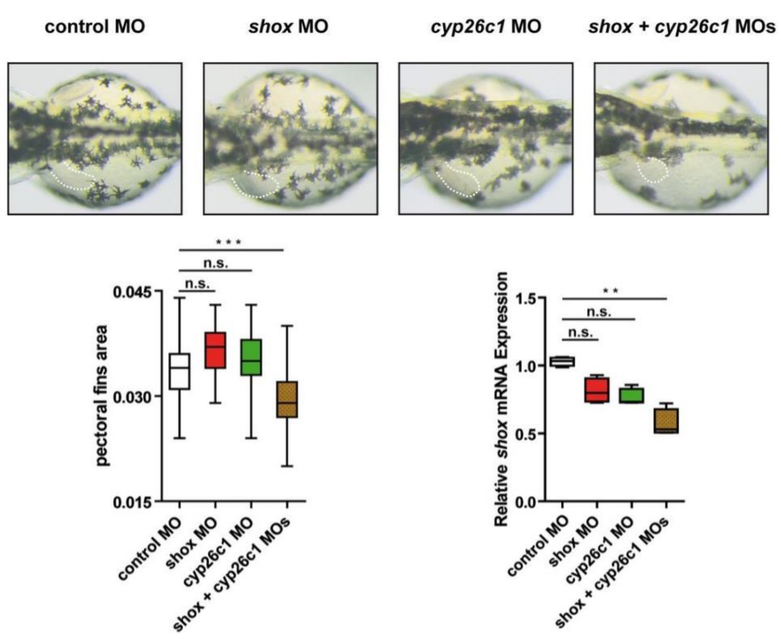

Figure 3. Modeling *SHOX* and *CYP26C1* interaction in zebrafish embryos
Different concentrations of *shox* and *cyp26c1* MOs were injected in zebrafish embryos to determine subphenotypic dosages. While single knockdown of either *shox* or *cyp26c1* using subphenotypic MO doses did not result in phenotype, double knockdown of *shox* and *cyp26c1* produced significantly smaller pectoral fins.

II. Idiopathic Short Stature

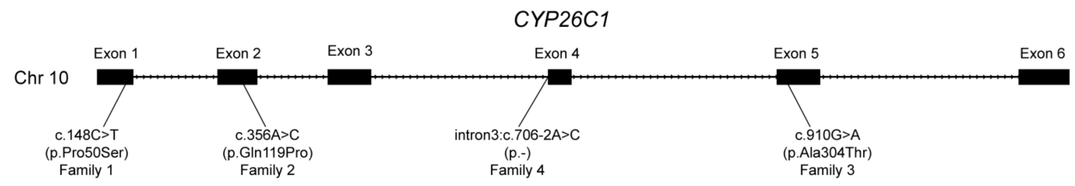

Figure 4. Schematic representation of the *CYP26C1* gene with identified variants

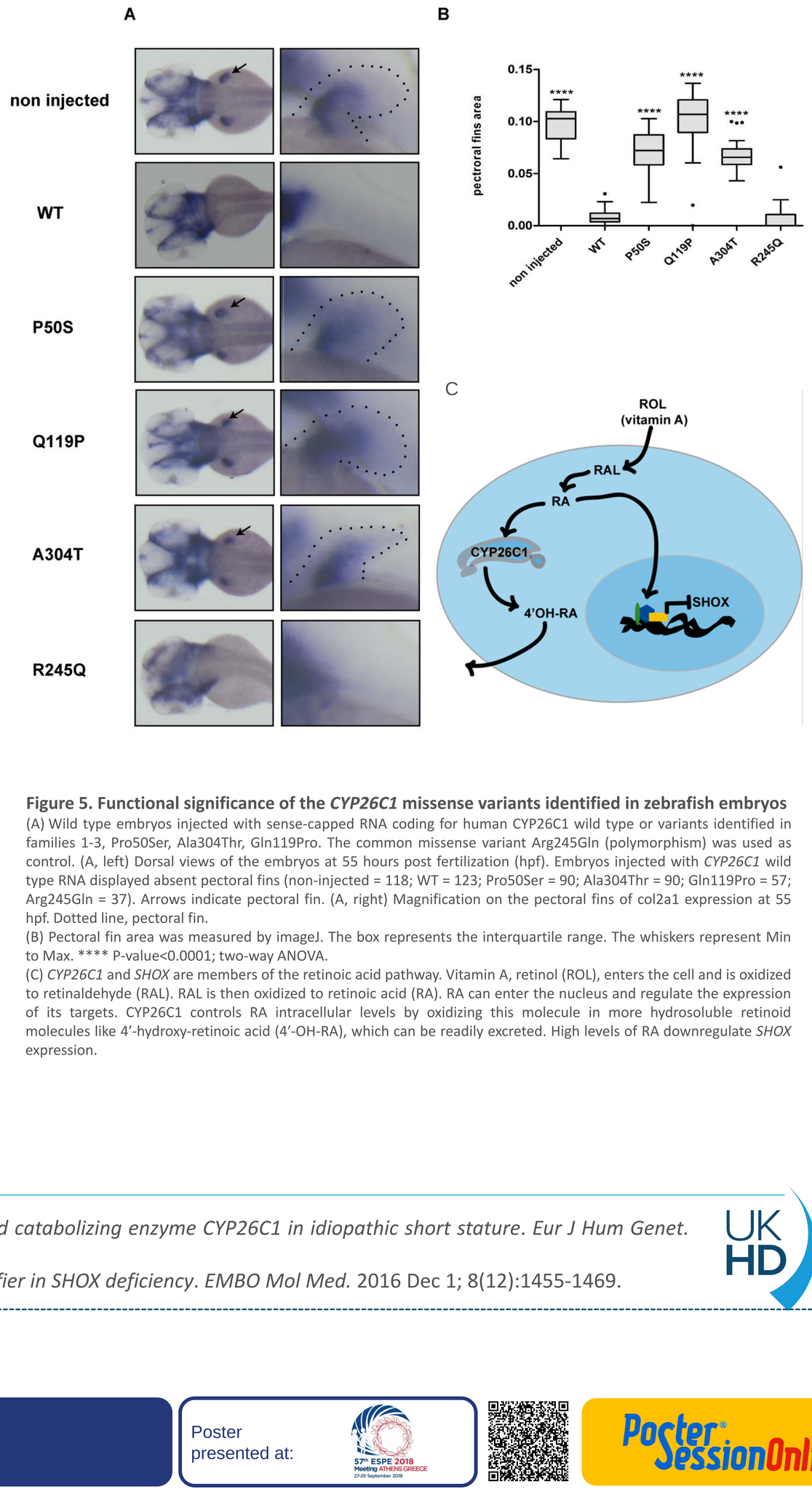

Figure 5. Functional significance of the *CYP26C1* missense variants identified in zebrafish embryos
(A) Wild type embryos injected with sense-capped RNA coding for human CYP26C1 wild type or variants identified in families 1-3, Pro50Ser, Ala304Thr, Gln119Pro. The common missense variant Arg245Gln (polymorphism) was used as control. (A, left) Dorsal views of the embryos at 55 hours post fertilization (hpf). Embryos injected with *CYP26C1* wild type RNA displayed absent pectoral fins (non-injected = 118; WT = 123; Pro50Ser = 90; Ala304Thr = 90; Gln119Pro = 57; Arg245Gln = 37). Arrows indicate pectoral fin. (A, right) Magnification on the pectoral fins of col2a1 expression at 55 hpf. Dotted line, pectoral fin.
(B) Pectoral fin area was measured by imageJ. The box represents the interquartile range. The whiskers represent Min to Max. **** P-value<0.0001; two-way ANOVA.
(C) *CYP26C1* and *SHOX* are members of the retinoic acid pathway. Vitamin A, retinol (ROL), enters the cell and is oxidized to retinaldehyde (RAL). RAL is then oxidized to retinoic acid (RA). RA can enter the nucleus and regulate the expression of its targets. CYP26C1 controls RA intracellular levels by oxidizing this molecule in more hydrosoluble retinoid molecules like 4'-hydroxy-retinoic acid (4'-OH-RA), which can be readily excreted. High levels of RA downregulate *SHOX* expression.

References: Montalbano et al., *Functional missense and splicing variants in the retinoic acid catabolizing enzyme CYP26C1 in idiopathic short stature*. *Eur J Hum Genet.* 2018 Apr; 26(8):1113-1120.
Montalbano et al., *Retinoic acid catabolizing enzyme CYP26C1 is a genetic modifier in SHOX deficiency*. *EMBO Mol Med.* 2016 Dec 1; 8(12):1455-1469.

57ESPE 175--P1
Growth and syndromes (to include Turner syndrome)
Gudrun Rappold
Poster presented at: 57th ESPE 2018 Meeting ATHENS GREECE 27-29 September 2018