mRNA expression profiling of key drug disposition genes in developing zebrafish until the juvenile stage

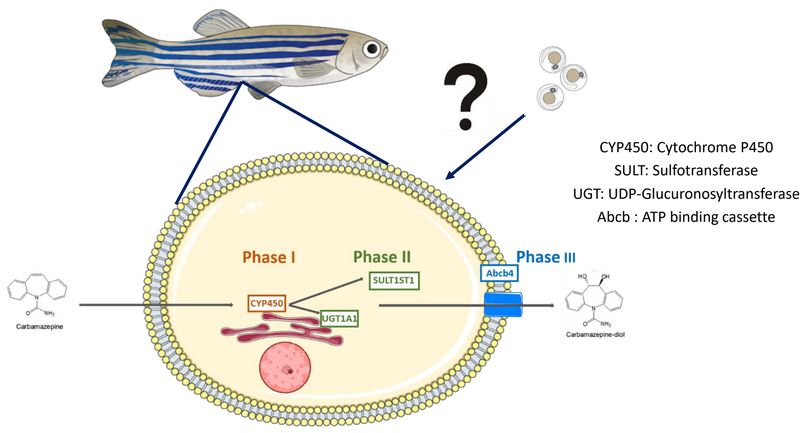
Chloé Bars[1], Evy Verbueken[1], Jelena Periz-Stanaćev[2], Lucia Vergauwen[2], Ellen D.G. Michiels[2], Evelyn Stinckens[2], Isabelle J. Gabriëls[2], Dries Knapen[2], Chris Van Ginneken[1], Steven Van Cruchten[1]

[1]Applied Veterinary Morphology, Department of Veterinary Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[2]Zebrafishlab, Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

Corresponding author: tel.: + 32 (0)3 265.24.00 ; e-mail: Chloe.bars@uantwerpen.be

INTRODUCTION

The **zebrafish model** has become an intermediate in toxicological research between *in vitro* models and *in vivo* mammalian models. However, the **biotransformation capacity** of the **embryo** is still questioned. No maternal metabolism is present in this model and this could lead to false negative results during the screening of xenobiotics for developmental toxicity.



CYP450: Cytochrome P450
SULT: Sulfotransferase
UGT: UDP-Glucuronosyltransferase
Abcb : ATP binding cassette

The aim of the study is to thoroughly **characterize the ontogeny** of **phase I**, **phase II** metabolizing enzymes, and a **phase III** drug transporter (Abcb4) in zebrafish embryos and larvae up until the juvenile stage.

METHODS

- **qPCR:** 25 time points from 0.06 to 32 days post fertilization (dpf). Four biological replicates/time point, each replicate formed by a pool of 10-30 (depending on the age) whole organisms. RNA extraction and qPCR using SYBR green on an Mx3005P instrument (Agilent Technologies).

- **Fluorimetry:** 200 µg/ml of microsomes prepared from whole organism homogenates (embryos, larvae and adults) exposed to 1.2 µM of the fluorogenic substrate benzyloxy-methyl-resorufin (Vivid® BOMR Substrate). Measurement with Tecan Infinite® 200 PRO microplate reader.

CONCLUSIONS AND PERSPECTIVES

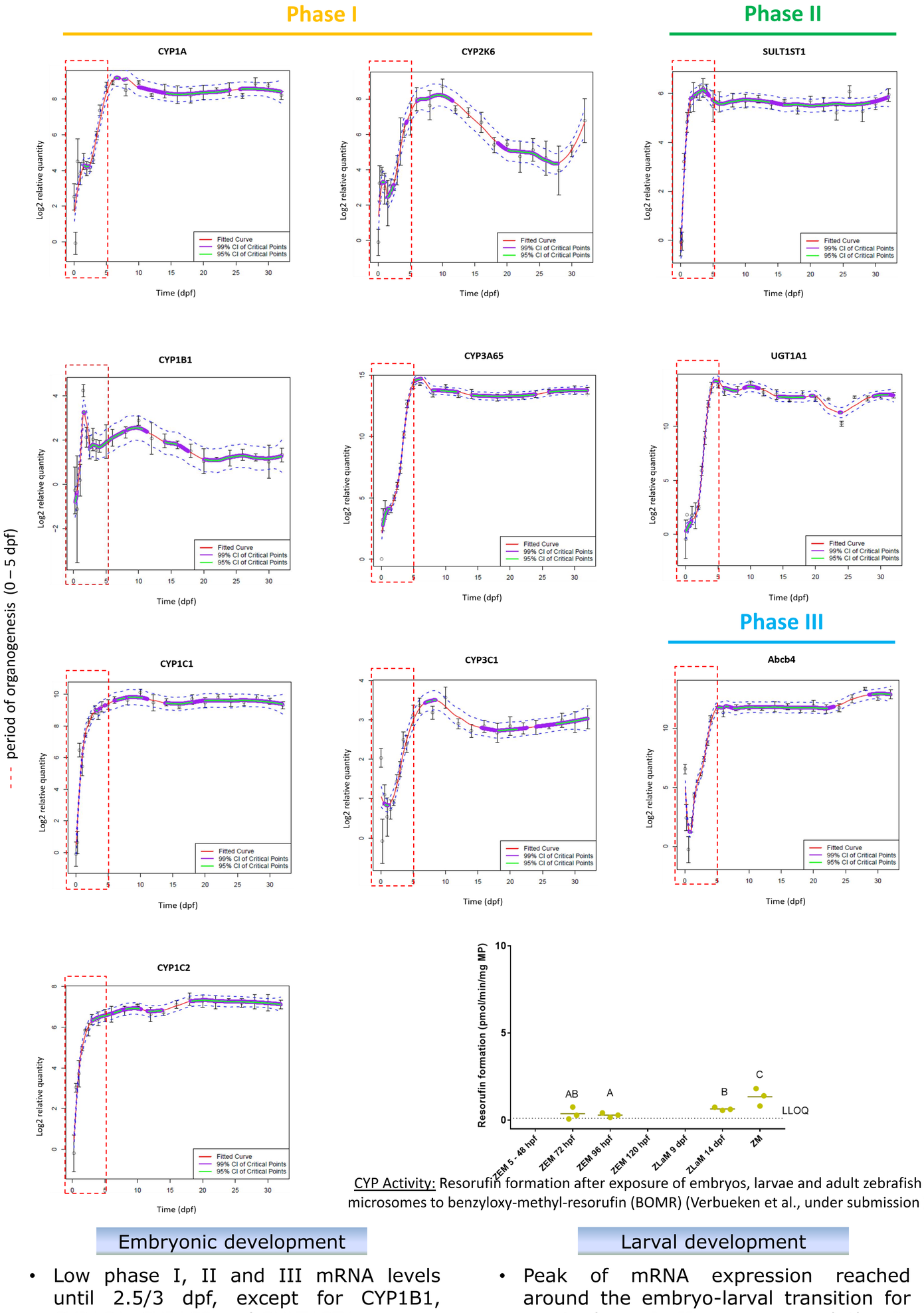
- Until 3 dpf, **low mRNA expression of drug disposition genes,** except for CYP1B1, CYP1C1, CYP1C2 and SULT1ST1.

- mRNA expression of these different drug disposition genes seems to reach a **peak at the end of organogenesis** (around 5 dpf) and then stabilizes during the juvenile stage.

- **The CYP gene expression profile is in accordance with the *in vitro* CYP activity data**.

- Further characterization is needed by investigation **of the biotransformation of human proteratogens** in zebrafish by using molecular and LCMS methods.

RESULTS AND DISCUSSION



CYP Activity: Resorufin formation after exposure of embryos, larvae and adult zebrafish microsomes to benzyloxy-methyl-resorufin (BOMR) (Verbueken et al., under submission)

Embryonic development

- Low phase I, II and III mRNA levels until 2.5/3 dpf, except for CYP1B1, CYP1C1, CYP1C2 and SULT1ST1.

- A steep increase between 3 and 5 dpf, except for CYP1B1.

- Maternal transfer for CYP1A, CYP3C1 and Abcb4.

- The gene expression profile is in accordance with *in vitro* CYP activity, as observed in previous work (Verbueken et al., under submission)

Larval development

- Peak of mRNA expression reached around the embryo-larval transition for most phase I, II enzymes and the P-glycoprotein.

- Stable mRNA expression throughout the larval period except for the CYP1B1, CYP2K6, CYP3C1 and UGT1A1 where fluctuations are observed.

- High relative quantity levels for CYP3A65 and low levels for CYP1B1 and CYP3C1.

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