Induction period of parasite-induced cholangiocarcinoma in humans

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1. Context

First infection with the foodborne liver fluke *Opisthorchis viverrini* typically occurs in childhood in endemic regions of Southeast Asia, yet cases of the resulting cholangiocarcinoma are observed decades later (Fig. 1). The epidemiological link between parasite exposure, which is dynamic over a person's lifetime, and the timing and probability of carcinogenesis are poorly understood in humans [1].

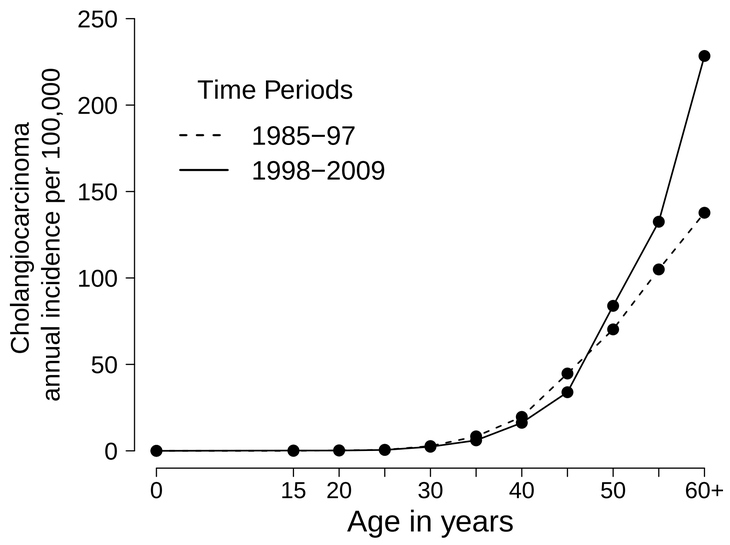

Figure 1: CCA incidence by age in Northeast Thailand

In this study, we aimed to infer the induction period in humans, defined as the time between first parasite exposure and the onset of driver mutations.
Firstly, we characterised exposure to *O. viverrini* infection using mechanistic models fitted to four pre-intervention parasitological surveys between 1980–1989 in N.E. Thailand (n=1,931), and the subsequent decline in parasite transmission from five later surveys (n=8,213) following a national control program (1987–1991) [2].
Secondly, we applied evolutionary models to whole-genome sequence data from 22 cases of cholangiocarcinoma from N.E. Thailand [3] to infer the timing of driver mutations.

2. Exposure to the parasite

Before the onset of large scale control programs in Thailand, we estimate that the average age of first infection with *O. viverrini* was 1.3 years of age (90% prediction interval [PE] 1.2–1.6 years) in endemic regions. After the onset of public health interventions the average age of first infection rises to 8.5 years (90% PE 7–12 years) (Fig. 2).

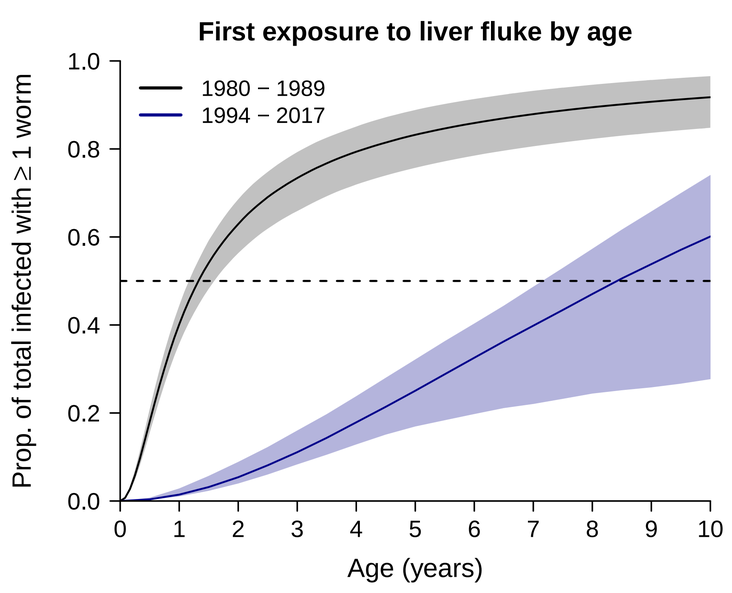

Figure 2: Average age of first exposure in surveyed areas of Thailand before (1980–1989) and after (1994–2017) a national control program. The solid lines show the median values, and the shaded areas are 90% prediction intervals

The mean cumulative number of *O. viverrini* parasites that a person had been exposed to by age 25 is 360 worms in the pre-intervention surveys (90% credible interval [CrI] 280–460 worms), and $\geq$ 1010 in the top decile of infected people. In the post-intervention surveys, the cumulative number of worms by age 25 fell to 2 worms (90% CrI 1–4 worms), and $\geq$ 8 worms in the top decile of infected people.
We estimate the lifespan of *O. viverrini* worms within our model as 8.5 years (90% CrI 5.6-14.1 years), which is defined as the average time for half of adult stage parasites to die within the human host in the absence of anthelmintic treatment.

3. Timing driver mutations

Using 22 whole–genome sequences of tumours from fluke–associated cholangiocarcinoma patients from N.E. Thailand [3], we called somatic single nucleotide variants, copy number alterations (Fig. 3), and inferred the clonal status of these mutations. We then applied evolutionary models (AmplificationTimeR and MutationTimeR [4]) to estimate the timing of driver mutations.

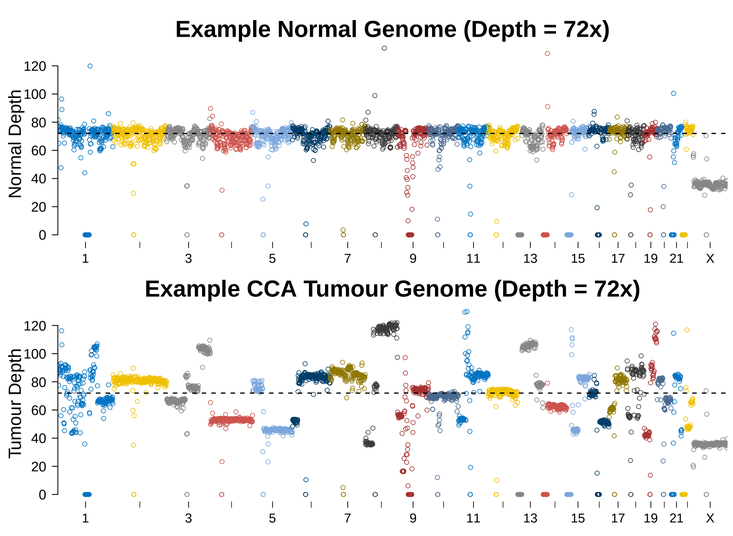

Figure 3: Genome-wide depth plot for cholangiocarcinoma (CCA) sample TH9

In 12 individuals with sufficient ploidy and tumour purity to infer the timing of genome amplifications, the earliest driver mutations occurred between 6–59 years, with an median age of 25 (Fig. 4). The most common mutations were in genes *TP53* and *KRAS*.

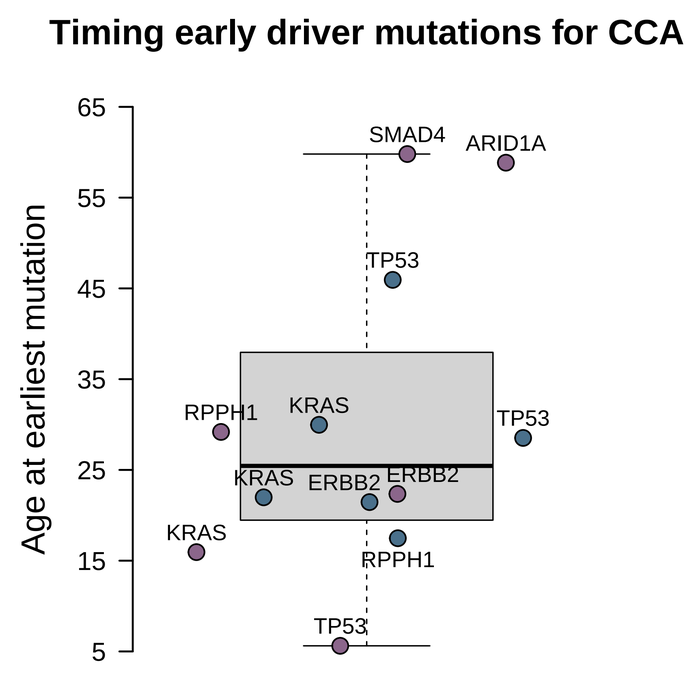

Figure 4: Age of the earliest mutation for 12 fluke-associated cholangiocarcinoma patients from Thailand. The earliest driver mutation gene is labelled. Points are coloured by patient sex (female=lilac, male=blue).

4. Conclusions

The induction period between first infection with *O. viverrini* and the onset of driver mutations for cholangiocarcinoma was a median of 24 years (range 4–58 years). Our estimate is most relevant to people born before, or during, the 1980s in N.E. Thailand. Within this population, the most exposed decile had been cumulatively infected with >1,000 *O. viverrini* worms by 25 years of age. The latent period, defined as the median time between the first driver mutation and hepatic surgery, was 31 years (range 9–50 years). Our results highlight the need for enhanced anthelmintic treatment among individuals <30 years of age, and subsequent monitoring for somatic driver mutations.

References

[1] Crellen, T. *et al.* Towards evidence-based control of opisthorchis viverrini. *Trends in Parasitology* **37**, 370–380 (2021).

[2] Jongsuksuntigul, P. & Imsomboon, T. The impact of a decade long opisthorchiasis control program in northeastern thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health* **28**, 551–557 (1997).

[3] Jusakul, A. *et al.* Whole-genome and epigenomic landscapes of etiologically distinct subtypes of cholangiocarcinoma. *Cancer discovery* **7**, 1116–1135 (2017).

[4] Gerstung, M. *et al.* The evolutionary history of 2,658 cancers. *Nature* **578**, 122–128 (2020).