UDP-glucuronosyltransferase (UGT) in Pinniped species can be analyzed with the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool to predict pseudogenes in other species

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Introduction

- The US Environmental Protection Agency (EPA) is evaluating alternatives to animal testing, making computational tools that evaluate chemical safety and cross species susceptibility both important and necessary
- The US EPA Sequence Alignment to Predict Across Species Susceptibility tool (SeqAPASS v6.0; https://seqapass.epa.gov/seqapass/) may be used with existing data to predict possible pseudogenes as determinants of species susceptibility to chemicals
- UDP-glucuronosyltransferases (UGTs) are mainly found in the liver and aid in metabolizing of drugs or other chemicals
- Pseudogenes are genes that have mutated into an inactive form and no longer have function

Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS)

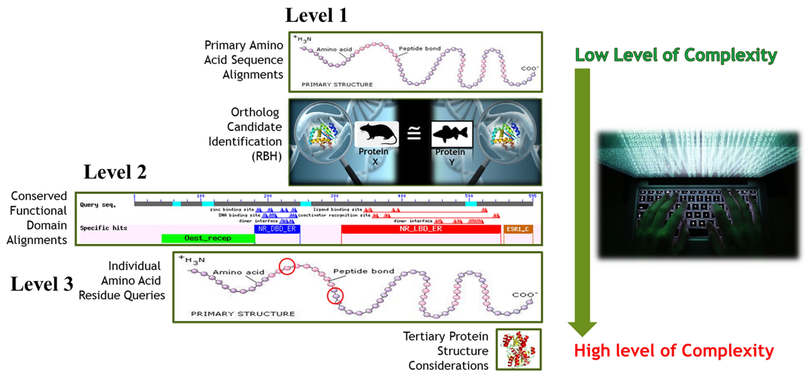
Understanding Protein Conservation

1. Must know the molecular target (e.g., pesticides)
2. Must identify a sensitive species

Chemical Molecular Target in Target Species ≅ Compare to Millions of Proteins From Thousands of Species

Greater similarity = Greater likelihood that chemical can act on the protein Line of Evidence: Predict Potential Chemical Susceptibility Across Species

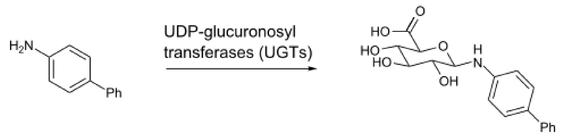
- SeqAPASS was developed to create a strategic and automated approach for assessing protein similarity to predict chemical susceptibility.



Case Example-UGT metabolism

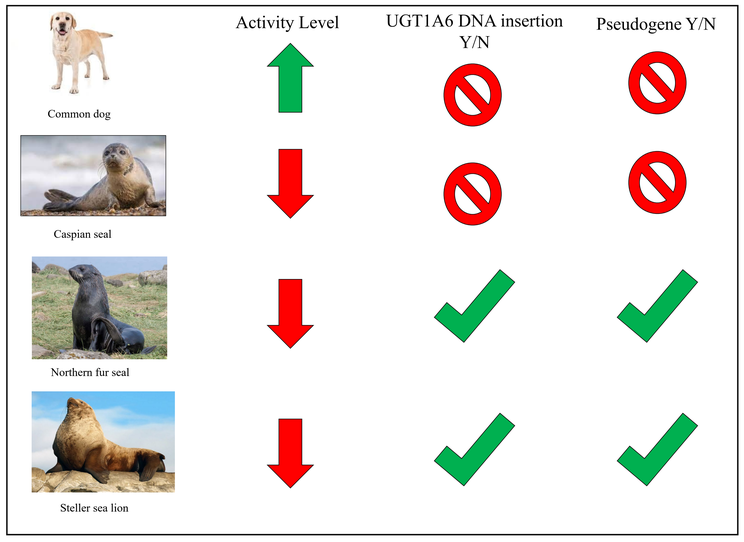
UDP-glucuronosyltransferase (UGTs)

- The most clinically relevant hepatic UGT isoforms are; UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15
- UGT1A6 involved in glucuronidation of acetaminophen and aspirin as well as other naturally occuring compounds
- Rat and dog shown to metabolize phenolic compounds due to functional UGTs
- Cats show low glucuronidation activity of drugs (e.g., acetaminophen) due to UGT1A6 pseudogene
- Pinnipeds are shown to have similar (low) glucuronidation activity to cats



UGT1A6

UGT metabolic activity and DNA sequence insertion



UGT1A6 Cont.

- Contain two mutation sites in UGT1A6 exon 1 for Northern fur seal and Steller sea lion where one site results in a premature stop codon
- Metabolic activity of the Caspian seal was low but the UGT1A6 is not a pseudogene
- Low glucuronidation activity in Caspian seal could be due to low counts of UGT isoforms

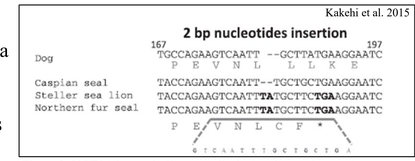


Figure 2: UGT1A6 exon 1 alignment displaying the two base pair nucleotide insertion for Steller sea lion and Northern fur seal resulting in a premature stop codon for both species

SeqAPASS Results

- Dog, Caspian seal, and Harbor seal UGT1A6 are all deemed functional proteins. Steller sea lion and Northern fur seal are pseudogenes

Total Match | Partial Match | Not a Match | Susceptible Yes | Susceptible No

Common Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5	Amino Acid 6	Amino Acid 7	Amino Acid 8
Dog	Y	58E	59V	60N	61L	62L	63L	64K	65E
Caspian seal	Y	29E	30V	31N	32L	33L	34L	35K	36E
Harbor seal	Y	58E	59V	60N	61L	62L	63L	64K	65E
Steller sea lion	N	29E	30V	31N	32L	33C	34F	35R	36N
Northern fur seal	N	29E	30V	31N	32L	33C	34F	35R	36N

Side Chain Classification: acidic, basic, aromatic, *etc*.
MW as surrogate for size: > 30g/mol different size
Susceptibility different than template = Both Class and Size Differ

Figure 3: An example of a SeqAPASS Level 3 Heat Map simple report showing amino acid susceptibility predictions utilizing colors to denote Total Match, Partial Match, Not a Match

Summary and Conclusions

- Amino acid position 63 (dog) could be used to possibly identify pseudogenes
- Kakehi *et al.* 2015 identified differences in gene numbers, for UGT1A isoforms in species, that could contribute to decrease in xenobiotic metabolism
- Based off sequence comparison, pseudogenes are uncommon and xenobiotic metabolism differences are most likely caused by differences in protein count
- Extensive knowledge of UGT differences between species could allow for further extrapolation of data with the SeqAPASS tool

References

1. **LaLone *et al.* 2016**, SeqAPASS: A Web-Based Tool for Addressing the Challenges of Cross-Species Extrapolation of Chemical Toxicity. *Toxicol Sci,* 153 (2): 228-245 (2016)
2. **Kakehi *et al.* 2015,** Uridine Diphosphate-Glucuronosyltransferase (UGT) Xenobiotic Metabolizing Activity and Genetic Evolution in Pinniped Species. *Toxicol Sci,* 147 (2): 360-369 (2015)