it is a great privilege to have dr ken

ken received his phd in pharmaceutical

science from the university of

washington in 9 and completed a

postdoctoral fellowship in pharmacology

at the university of connecticut

in 99 he joined the faculty of the

university of washington school of

pharmacy and was promoted to the rank of

full professor in 00

until a couple of years ago he was the

chairman of the department of

pharmaceutics

kens research interests include

elucidating of genetic hormonal and

environmental factors that contribute to

interindividual differences in

xenobiotic biotransformation

ken is a fellow of aas

and aaps and the recipient of the ross

palmer progress and medicine award from

ascpt

hes a past president of the american

society of pharmacology and experimental

therapeutics im confident youre going

to enjoy todays lecture

in this lecture i will introduce

um the the topic of phase one metabolism

and its role in the elimination of

pharmacologically active uh drugs and

and metabolites ill also be touching on

mechanisms that underlie

interindividual differences in the

metabolic processes that contribute to

variability and drug efficacy and drug

safety

all of of critical importance to the

provision of therapeutic

drug therapy in

the treatment and prevention of disease

first lets consider this scheme here

which a compartmental model of drug

elimination

following intravenous as well as oral

administration

following intravenous administration

drug is of course introduced into the

venous

blood system circulation

travels to the heart and lungs and then

passages into the arterial supply

arterial blood coming out of the heart

perfuses all organs of the body

including those that are are mediating

the pharmacological effect as well as

the organs of elimination and the two

principal organs of elimination are the

kidney and the liver

drug blood

will distribute drug into both of these

organs where it then can be acted on and

either metabolized by drug metabolizing

enzymes or excreted through filtration

or transport processes in the kidney of

course we have the the option of

filtration some drug of course can be

reabsorbed but much can be eliminated

into the urine there are also

transporters both on the the

the

vascular as well as the luminal sides

that can facilitate direct excretion of

drug into the filtrate the kidney has

very little metabolic activity in terms

of the ability to clear drugs there are

some enzymes that are expressed there

but primarily it is a organ of drug

excretion

in contrast the liver has both metabolic

as well as

excretory

activity uh drug partitioning into the

liver

can undergo metabolism by a variety of

enzymes including the important phase

one enzymes parent drug of course can be

excreted into the bile but generally it

must undergo some biotransformation

first before it ultimately is excreted

in into the bile or back into the blood

for elimination through the kidney

following oral administration

you have those same two organs each of

the elimination of the drug but in

addition you have contributions of the

intestine to the elimination first pass

elimination of drug uh following its

administration into the gut movement

drug that is is dosed into the lumen of

course has to um

be solubilized and then free drug can be

partitioned into the intestinal

enerocytes within the anaerosites it can

undergo metabolism

to products that then either may be

absorbed into the blood or excreted into

the feces along with any drug that isnt

absorbed

of course drug has a

opportunity to be absorbed into the

microvasculature in the intestine

that feeds into the portal vein and then

distributes into the liver for another

round of potentially first pass

elimination

drug that escapes elimination is

absorbed and escapes elimination in the

intestine in the liver uh will of course

then be delivered via the venous

circulation to the heart and then

circulating around for another round of

systemic

elimination processes by the kidney in

the liver

so the key distinction between iv and

oral dose elimination is the role of the

intestine in first pass elimination as

well as the liver in contributing to

that first pass loss before drug

is delivered to the rest of the body to
exert its pharmacologic effects and both
drug metabolizing enzymes in particular
phase one enzymes in the intestine and
the liver as well as the transporters
all contribute to the elimination of
drug from the body
thinking then about the relative roles
of the intestine and and liver and the
kidney to drug elimination we can think
about then the primary routes of
elimination and the contributions of the
the liver as as well as the kidney to
the elimination of drugs
and this is a data that was collected a
few years back its still pretty
relevant though looking at the top 00
drugs
by prescriptions and the predominant the
major route of elimination of a drug all
drugs are eliminated by multiple
processes but typically one process is
going to dominate and of those top 00
drugs one can see
that the primary
predominant route of elimination is

through hepatic

elimination

the kidney

contributes to dominates the clearance

of about of the drugs but the

majority arent going to be eliminated

primarily by by the liver and within the

liver the predominant route of

elimination is through metabolism

that is you have

contributions to the biotransformation

of drugs by the phase cytochrome p0

enzymes which well be talking about in

great detail

other phase enzymes some phase

enzymes conjugative enzymes and then in

this context here listed as unknown with

probably some that involve biliary

excretion direct biliary excretion

of the drugs that that are metabolized

in the liver by the phase one processes

these are principally catalyzed by the

cytochrome p0s and the enzymes that

are most important in this phase one

biotransformation of drugs are listed

down here the cytochrome p0s a

dominating on the the phase one enzymes

as well as contributions from

uh c9 d c9 and na these are all

the principal enzymes involved in phase

metabolism of drugs that are

administered by either iv or

oral administration

thinking then about the liver and its

role in in drug elimination and and

considering it as a clearance process

drug of course must be taken up into the

hepatocytes and um uptake into the

apatocites um

by either passive processes or active

transport through through the um if you

will the the sinusoidal uh transporter

showed here as a is a phase zero process

um this this categorization of the

different processes of hepatic clearance

uh was was uh developed a number of

years ago and that day zero involves the

uptake by either passive or active

mechanisms

once in the hepatocyte on the drug

depicted here as a p for parent molecule

typically will undergo some phase one

bowel transformation we consider this

process a biotransformation to be a

functionalization process in that its

its basically imparting on the molecule

sure that allows it then most often to

undergo conjugation by phase two enzymes

such as the ugts or the sulfur

transferases

drug that then under has been

metabolized to a a primary metabolite

and then to a secondary conjugated

metabolite will be transported out of

the hepatocyte it is typically too polar

to be diffusing across the cell membrane

and must be transported and that can

either be transported into the bile by

the the catalytic transporters or into

back into the if across the base lateral

membrane for ultimately release into the

vasculature

by other other um transporters

that excretory process is considered to

be phase three or the eflux out of the

hepatocyte

and for this particular talk were going

to be focusing our attention on the

phase one processes that

functionalization

if you will of drugs into metabolites

that can either be excreted directly or

undergo conjugation to a secondary

metabolite for ultimate excretion

thinking then about the this um process

of hepatic um biotransformation and

excretion we can apply a mathematical

concepts to

the efficiency of those particular

processes and importantly thinking about

its the contributions of those processes

to hepatic clearance as well as the

bioavailability of a drug following oral

administration

depicted here is basically the process

of drug absorption

into from an oral delivery in the

intestinal lumen into the anaerosites

where it can undergo metabolism by

enzymes

particularly phase one and phase two

enzymes in the anaerocytes for

ultimately elimination

through the feces or absorption into the

blood

unchanged drug that gains access into

the portal vein and escapes that first

past metabolism by the enterocytes will

passage into the liver and of course

undergo metabolism again or

basically release into the systemic

circulation

and one can define then the fraction of

a dose that actually escapes is absorbed

and escapes first pass elimination in

both the enero sites in the liver by

this mathematical relationship where

that fraction or the bioavailability

observed is is equal to the area under

the curves of the concentration time

profiles following oral administration

over intravenous administration times

the relative doses theyre administered

from

the availability perspective that

availability observed is equal to the

products of three different terms the

fraction of a dose that is actually

absorbed into the enerocytes

the fraction that escapes

first past metabolism fg in the

enterocytes and then the fraction that

escapes a hepatic metabolism or

excretion in the liver

and for each of these organs in

particular the liver one can define that

availability as simply one minus the

extraction ratio that of the the dose

that is delivered into the organ and

that

undergoes elimination

thinking about each organ in particular

the liver one can define the clearance

of that uh metabolic clearance and total

clearance of the of the

liver enzymes and excretory transporters

with this relationship here clearance is

assumed to be the sum of individual

processes that are acting on drug within

the nra site all those processes are

assumed to operate in parallel and thus

the clearance terms are are additive

within the hepatocyte then um within the

the considering the total body clearance

here the total body clearance is equal

to the sum of those clearances within

the liver

as well as the the the clearance in the

kidney by the kidney and then clearance

potentially by any other um organ of of

the body these being the two principal

clearance organs

considering the liver clearance that

hepatic clearance one can define

the efficiency of that process by this

relationship here where you have drug

being delivered into the liver via

either the portal vein or the hepatic

artery and eliminated by

the hepatic vein for distribution into

the to the vet to the venous circulation

and the rest of the body within the

liver metabolism and secretion can occur

and that clearance that efficiency of

the elimination in by the liver is equal

to the product of the extraction ratio

times the blood flow going into that

organ and that could be further defined

by this relationship as the product of

hepatic blood flow times the product of

the fraction unbound and the unbound

intrinsic clearance

for the elimination process

and that term there that product is

divided by the sum of blood flow plus

that same product of the fraction

unbound and the unbound intrinsic

clearance

the ultimate efficiency of elimination

then is defined by both blood flow as

well as the efficiency the intrinsic

clearance of either the metabolic

enzymes or the secretory enzymes in this

lecture were going to be focusing our

intention on the metabolic enzymes the

metabolic processes that are catalyzed

by phase enzymes

the intrinsic clearance that unbound

intrinsic clearance within the liver the

total unbound intrinsic clearance is

simply the sum of the individual

intrinsic clearances for each metabolic

process each enzyme that is catalyzing

the elimination of drug

and for each of those enzymes one can

further express them in terms of their

michaelismenten

constants shown here vmax over the km

assuming nonsaturable kinetics

and that total intrinsic clearance is

simply the sum of the individual

intrinsic clearances associated with

each enzyme that can catalyze the

elimination of the drug

and for each of those intrinsic

clearance they can be further broken

down

the v max term as the product of the

total enzyme pool times the kcat for

that elimination process divided by its

km

so the total intrinsic clearance is

simply then the sum of these michaelis

metin

ratios constant ratios

for each metabolic or secretory process

and so within the liver then there are

multiple enzymes and some of those maybe

only one maybe two maybe three will be

involved in the the biotransformation of

a drug that is presented into that organ

the enzymes that are available for

metabolism are are are quite um

large there are multiple different

enzymes the phase one enzymes being the dominant ones and the cytochrome p0s being the dominant phase uh one enzymes the liver though contains other drug metabolizing enzymes in fact almost all of the enzymes that are found in the human body can be found in significant concentrations within the liver a few exceptions sip a an enzyme that is expressed predominantly extrapolate as well as a couple of the ugts that are found only within the intestinal enterocytes the intestine has a considerable array of drug metabolizing enzymes including these phase one enzymes up here the cytochrome p0s however the dominant form that is expressed in the intestine is the sip a form sip a and ca with a little bit of contributions from the c forms c9 and c9 other enzymes are expressed only weekly except perhaps sip a which can be expressed in significant levels following exposure to polycyclic aromatic hydrocarbons which are typically found

in cigarettes of smoke so cigarette

smoking can induce sipa in the

intestine the other enzymes that are

found within the intestine are the ugts

um several of the form major forms of

the ugta family as well as to the b

family

and of course other conjugating enzymes

the sulfur transferases glutathione

stransferases the gnats and the

important carboxyl esterases which act

on prodrugs that then must be

hydrolyzed to release active molecules

that then are delivered to the body for

their pharmacological effects

thinking then about each uh cell and and

the localization of these

phase one enzymes within the enterocyte

or the hepatocyte

one can

look at this particular illustration

here to to identify within the cell the

organelles in which these enzymes are

concentrated and for the cytochrome

p0s as well as as fmo and other phase

enzymes these are predominantly

localized within the endoplasmic

reticulum

the endoplasmic reticulum of course is

also referred to as the microsomal

fraction

this is a

basically sphericals of the er that are

formed following uh disruption of the

cell membrane on isolation

um

those microsomes contain the p0s as

well as fmo and some of the carboxylase

races and even some of the conjugating

enzymes such as eugets

the other site of drug metabolizing

enzymes is cytosol basically the aqueous

environment that sits um that that

perfuses the entire um in intracellular

domain and within the cytosol you have

other phase one enzymes like aldehyde

oxidase but also conjugating enzymes

like the sulfur transferases and the

anacetyl transferases

mitochondria typically doesnt have drug

metabolizing enzymes um sorry shown here

and but um what is found in there is the

the monoamine oxidases which is

sometimes involved in drug metabolism

but but infrequently

the predominant

bulk of the drug metabolizing activity

is going to be catalyzed by the

cytochrome p0s and those are found

within the endoplasmic reticulum

fraction

so lets say some more about the

cytochrome p0 since that is the major

route of drug metabolism for drugs that

are administered to patients it is a par

the cytochrome p0s are a super family

of gene products there are of these

in in humans the highest concentrations

of the cytochrome p0s in general total

concentrations are found within the

liver

however if one thinks about specific

forms say such as sip a one can find

equivalent levels with equivalent

concentrations of that particular

p0 isoform in both the liver and the

small intestine

the name cytochrome p0 is derived from

a unique absorption

spectrum a different spectrum

following

the reduction one electron reduction of

the iron in the in the hemoprotein of

the p0 and complexation with carbon

monoxide it gives a a maximum at 0

nanometers um and a minimum at about 0

nanometers

this um characteristic absorption

difference spectra um actually reflects

the abundance of the p0 the p if you

will the difference between 0 and 00

nanometers

is is proportional to the concentration

of the p0s that are forming this

particular complex

the function of the p0

is defined by its localization in the

endoplasmic reticulum

it sits on top of the membrane anchored

by an nterminus

shown depicted here in this helix here

but the majority of the enzyme actually

sits on top of the er membrane facing

the cytosol and therefore

drug can access that particular enzyme

in in one of two ways the dominant way

for most lipophilic drugs that are given

orally is actually partitioning into the

membrane and then accessing the active

site the iron of the heme protein

through this substrate access channel

once biotransformed by the enzyme

in in the active site the product then

will egress

basically through a an aqueous channel

and then released into the cytosol for

further disposition

the function of this this heme protein

here is is requires essential

contributions from the cytochrome p0

reductase cpr which is an nadph

dependent coenzyme that basically

delivers electrons from nadph to the

cytochrome p0 heme iron

and to to catalyze the biotransformation

of xenobiotics

so lets say some things then about

those biotransformation reactions the

cytochrome p0s can catalyze a wide

variety of oxidation reactions but

typically these are oxidations that are

occurring on carbon but you can catalyze

the oxidations of other

molecules nitrogen and oxygen

and sulfur as well

considering then the biotransformation

of molecules

such as dextromethorphan shown here

cytochrome p0s will catalyze an

oxidation of the

um

carbon mo adjacent to a heteroatom such

as nitrogen and oxygen

that hydroxylation reaction once it

occurs results in spontaneous release of

of in this case here formaldehyde

releasing the free amine

of the dextromethorphan molecule

cytochrome p0

a

and this in predominant enzyme

catalyzing this particular nd alkylation

reaction

another p0 sipd catalyzes an o

demethylation reaction that is initiated

by oxidation of the alpha carbon

adjacent to the oxygen atom

these nd alkylation and od alkylation

reactions are very common they found for

many drugs that are therapeutic agents

that are used to treat diseases

another common type of oxidation

reaction that p0 enzymes can catalyze

is the the hydroxylation of aromatic

molecules typically in the para position

to the substitution so one can see as

shown here in the parahydroxylation of

diphenyl hydantoline or phenytoin

a reaction that is catalyzed

predominantly by sipc9 with a little

bit of contribution from sipc9

again aromatic hydroxylation is a very

common

type of biotransformation reaction

in this particular example here with

phenytoin pair hydroxylation it

illustrates the regioselectivity that is

the para position its the

preferred site of hydroxylation and then

it generates a chiral metabolite

here as a result of stereocell

biotransformation

the enzymes cytochrome p0s in in found

within the liver

have been studied extensively over the

the last three to four decades and it

has resulted in a compilation of

an identification of molecules that are

selective or diagnostic substrates as

well as inhibitors of the key p0

enzymes that are involved in drug

biotransformation

these diagnostic substrates and

inhibitors are in powerful tools for the

pharmaceutical industry

in their attempt to characterize both

the disposition of their particular new

molecular entity as well as its

potential to cause drug drug

interactions

and these diagnostic substrates and

inhibitors are used to first

characterize the contribution of

different p0s to the metabolic

elimination of a new molecular entity by

selective inhibition of individual

isoforms in a say um

system that includes all of the p0s of

of importance say for example human

hepatocytes are human liver microsomes

so each of these inhibitors can be used

to identify the contribution of a

different form to the metabolic

elimination of a new molecular entity

the diagnostic substrates shown here

some such as s warfarin for c9

midazzlam for a dextromethorphan which

we just talked about for d can be used

to identify whether the new molecular

entity has the propensity to cause a

drug interaction either inhibition of

these individual cytochrome p0 forms

or in fact induction of those cytochrome

p0 forms so these diagnostics

substrates and inhibitors of the p0s

again are powerful tools for the

characterization of both the metabolism

as well as the ability to cause drug

interactions of new molecular entities

i want to say a few things about p 0

taxonomy

um the the nomenclature rules um so that

theres an understanding of how the

different isoforms were were described

when describing any p0 gene product

say d for example the sip designates

that its a cytochrome p0

the first arabic numeral here identifies

or designates the p0 family in which

it is found

and then the capital editor that follows

designates the subfamily and finally the

last numeral here distinguishes the

individual gene product

within a particular sub family

now classification within families and

sub families

is is based on amino acid sequence

homology how similar are two p0

isoforms

amino acid sequence to each other

p0 sequences that are are greater than

0

are going to be found within a given

family if theyre less than 0 percent

then theyre going to be placed into

different families

um so its possible that a p0 could

end up within a unique family all by

itself if it were any had no similarity

to another the drug metabolizing p0s

in humans are all found within multi

gene families

and

families

classification within a subfamily is

based on a homology that is greater than

identity and within this particular

subfamily of the human p0s it can

range from that that limit of percent

to as to as as great as 9

amino acid sequence homology

and so they can have very very close

similarity or less similarity uh based

on those amino acid sequence

finally when thinking about the the fact

that there are genetic variations within

all the drug metabolizing enzymes

including the cytochrome p0s those

individual allylic variants are

designated by the star um

depicted here following the last numeral

and then a a numeral that indicates the

particular allele for that particular um

enzyme or gene product

in this instance here sip d a common

genetic variant is the star four variant

which results in loss of a function of

the p0 enzyme

saying some things about where the 0s

that catalyze drug metabolism in the

liver fit within the fulcrum compendium

of cytochrome p0s um

the three families that are involved in

drug metabolism one two three

um

are shown here but most of the

cytochrome p0s in the human body

actually catalyze the the same types of

oxidation reactions but of endogenous

substrates fatty acids vitamins bile

acids as well as steroids so most of the

p0s in humans are involved in this

endogenous biotransformation which have

important um

cellular functions and maintaining

hemostase

or homeostasis rather

and these three here are those that are

involved in biotransformation of

xenobiotics including drugs

each of the enzymes that are involved in

drug metabolizing including the

cytochrome p0s

exhibit significant interindividual

variability in terms of the abundance of

those enzymes within an individuals

liver or small intestine

that is basically a characteristic of

all the drug metabolizing enzymes is

this tremendous interindividual

variability

that variability is is a consequence of

two factors

a major source of it is the the

regulation of the genes that encode the

p0 proteins

this involves both constitutive

processes that is regulation by

molecules such as as

steroids

even growth hormone other hormones

throughout the body which exert effects

through modifying the activity of

transcription factors and and gene

transcription

the abundance of these enzymes drug

metabolizing enzymes can also be

influenced by xenobiotics which exert

their effects often through

activation of various transcription

factors

variability in the abundance of drug

metabolizing enzymes can also

be exhibited by posttranscriptional

mechanisms particularly the involvement

of micro rna which influences the

translation process and then less well

understood is epigenetic phenomena such

as methylation which can also regulate

gene transcription processes

and finally

there can be

variability in the the protein clearance

processes principally by proteasomal as

as well as lysosomal mediated uh protein

degradation

and then lastly of course we have

genetic variation which affects both the

synthesis of an enzyme as well as its

degradation which both can also

contribute to variability in enzyme

abundance

i wanted to show this example of the

variability in enzyme abundance is with

a major hepatic enzyme sypc9 this is

data collected from a combined liver

bank at the university of washington as

well as saint jude childrens research

hospital

depicted here is basically the frequency

of different abundances a sip c9

protein

expressed here as picomoles per

milligram of microsomal protein

quantified by an lcms ms method and you

can see here it is distributed in a

somewhat unimodal skewed pattern but

important to understand is the

quite variable

differences in the abundance of this

particular single p0 enzyme in human

livers ranging in this study from to

as high as 99 picomoles per milligram

over 0 fold variability in the

abundance of this single p0 amongst

different human livers and if this were

then translated in vivo one would expect

then the potential for

high variability in the clearance

metabolic clearance of drugs eliminated

predominantly by sipc9

and that is what is illustrated here

this is data that was published and

roland and poser derived from an earlier

publication depicting the relationship

between daily dose of this drug

phenotoid which undergoes

parahydroxylation by sipc9 and the

plasma phenytoin concentration trough

concentration achieved following

multiple dose administration so this is

essentially a steady state concentration

following a constant fixed dose that

varied from one patient to the next

based on other um and algorithms that

considered

other factors demographic factors as

well

as as concomitant medications and one

can see here that

the um

there is a dose if you will

concentration steady state concentration

relationship leads to higher systemic

blood levels of phenytoin but what what

should be appreciated is the fact that

at any given dose depicted with this

dotted line here theres tremendous

interindividual variability inter

patient variability and the blood level

achieved with the same dose administered

by multiple dose administration to a

patient

so that variability then is of course a

problem for

the clinician because in this context

here uh the the

safety and efficacy of penetrant is

defined by a narrow therapeutic window

of concentrations between 0 and 0

milligram per liter here and this

requires that

individualization of the dosing of

penetoine

to take into consideration this

significant interindividual variability

in the dose concentration relationship

now what drives this variability

well clearly in the case of phenytoin

nonlinear kinetics contributes to it

its a its a a drug that can saturate

the cypc9 enzyme and leading to a more

hyperbolic relationship between the

concentration

and and clearance process

but also contributing to this is of

course going to be genetic as well as in

environmental factors that will affect

that hepatic

sypc9 content

and so then thinking about the

metabolism and excretion of phenytoin

its fairly straightforward again here

the controlling factor though is sypc9

it catalyzes this initial

parahydroxylation leading to the

inactivation of phenotory and so the

pharmacology of phenytoin is controlled

by c9 and the interindividual

differences in its abundance and its

catalytic activity

secondarily there is conjugation by the

ugt enzymes a common process leading to

this conjugate here highly polar

conjugate that then can be excreted out

of the hepatocyte by mrp free

so

thinking then

in greater detail about those mechanisms

of interindividual variability this is

a nice illustration pointing out that

that variability will be dependent on

the genetic constitution what form of

the gene um is is present in any given

patient what variants are present and

how do they affect both the abundance of

the enzyme as well as its ability to

catalyze biotransformation reactions

in addition are

mitigating factors such as the age of

the individual patient the sex

for females are they pregnant or not

is there an infection is there

inflammation a cytokine releases that

can influence gene expression even

things like circadian rhythms throughout

the day can influence the transcription

of genes into their protein products and

of course the function of the liver

is is it is it dysfunctional or is it a

normal function

and then of course the administration of

xenobiotics um drugs that can influence

both the transcription as well as the

function of a a a enzyme a

biotransformation

and other factors even diet itself can

influence the the expression of genes

into their gene products all of these

factors as well as the genetic construct

constitution contribute to those

interindividual differences that were

illustrated in the phenotoid

dose

steady state concentration relationship

thinking a little bit more about how

genetic variation influences

both the the production of the protein

as well as its function

we consider the fact that for any given

gene its its a

defined by the presence of both exons

which are basically the codeine regions

of the gene um

defined by codons for specific amino

acids as well as introns in this case

here a simplistic view of three a gene

with three exons and two entrons

in addition there are flanking regions

both at the three prime region as well

as the five prime region

this particular gene then can be

transcribed by polymerases

those polymerases will will generate

heteronuclear rna which contains both

the

sequences for both the exons as well as

the introns

that heterorna is then

undergoes splicing by this glycosome to

convert it into a mature rna which now

um

basically encodes the particular protein

and that translation in the ribosome

proteins may or may not involve

posttranslational modification the

cytochrome p0s a key step here is the

incorporation of the heme to make the

enzyme fully functional

um

variation in the sequence here of a

particular gene at any site can

influence both the production of the

protein as well as its function as a

biotransformation enzyme and those

variants can do so by changing both the

structure of the protein that is

produced as well as the rate of

synthesis

the rate of synthesis controlled

primarily in the five prime region

by

domains referred to as promoters and

enhancer these domains basically respond

or are have an affinity for

transcription factors found within the

nucleus

those transcription factors are

activated by circulating molecules um

either either hormones or or xenobiotic

molecules other regulatory molecules

that bind to transcription factors and

then

associate with both enhancer and

promoter regions and drive the trench

the binding of the polymerase and the

transcription to a heterorna

theres also epigenetic loci that are

regulated by methylation that can

provide an overarching

regulation of gene transcription

so genetic variation within these

regions of the gene can also contribute

to variability in the transcription

process

lastly we also consider variation at the

three prime n in particular the three

prime utr

that is basically the binding domain for

micro rna micro rna are produced in all

cells including

the hepatocytes and those micro rna the

transcription the production of them is

dependent on environmental factors and

therefore variability in the environment

can influence the production of micro

rna and they basically influence the

translation efficiency of this mature

rna into the protein products

so these are all mechanisms by which you

can have both environmental factors as

well as genetic variability influencing

both the abundance of a protein and its

function

and to illustrate then some of that

genetic variation shown here is

basically the structure of sipc9 which

weve introduced and some of the common

variants the amino acid substitutions

coding variants that are found within

the human population

multiple different variants have been

identified at key amino acids depicted

here

the two uh common ones that are are

described and well described

are substitutions at the position

and arginine as well as substitution of

the isoleucine at 9 these two

variants lead to changes in the

efficiency of sypc9 and metabolizing

fanucoin as well as other substrates for

the enzyme

the star variant

exerts its effect primarily by

influencing the interaction

between the p0 and the p0 reductase

increasing the kd reducing that affinity

lowering the vmax of that reaction and

lowering the intrinsic clearance over

here the star variant has two effects

it it it affects the affinity of the

substrate for the enzyme increasing the

cariam it also leads to a lower v max

and again resulting in a lower intrinsic

clearance

that reduced intrinsic clearance is of

course then going to potentially

influence the elimination the metabolism

elimination of substrates for c9

two substrates commonly used diagnostic

substrates phenotoid and the production

of this product here para hydroxy uh

phenytoin as well as warfarin s warfarin

and the hydroxy

hydroxylation both catalyzed

predominantly by sipc9

this is data that was collected from

basically an experiment with

recombinantly expressed forms of the

cytochrome p0 the reference form the

star allele the star allele as well

as the star allele and those codeine

substitutions

and for both phenytoin and warfarin s

warfarin the

metabolism of these substrates is

altered in the um

the gene products from the star gene

variant as well as the star

influencing both the km and the v max

for phenytoin here you can see the star

causes a reduction in both in the v

max no change in the km whereas the star

um it results in a reduction in the v

max and an increased indication

thats the intrinsic its relative to

the reference form is reduced

for both the star and the star

variant with the star having the most

deleterious effects because of the

change in both km and vmax you can see

similar changes in the intrinsic

clearance towards warfarin through the

seven hydroxylation reaction

so this is the type of of

alteration in the intrinsic clearance of

substrates of septucc9 that can be found

within the human population

because of genetic variation

there are many other variants that are

found in in the human population some

that have been well studied shown here

also lead to similar reductions in both

the metabolism of both phenytoin as well

as as warfarin

even novel variants found in in work

that we conducted with alaska native

population also showing reduced activity

so genetic variation can be a major

source then of interindividual

differences in systemic concentrations

following multiple dose administration

and thats illustrated here in this

concentration time profile for phenotoid

and individuals who had been prior

characterization of their genotype the

concentration time profile of the

reference um

star one star one genotype is shown here

and then for individuals carrying either

one variant either star or star or

variants either being homozygous star

star or heterozygous star star are

depicted here

what should be apparent then is the the

consequence of genetic variation is an

increase in the systemic concentrations

and the auc of of phenotoid following

oral administration and this variability

then can lead to differences of course

in the safety and efficacy and necess

necessitate changes in dosing to um

basically

um

to to take into consideration the slower

clearance of phenotoid in these

individual patients

and that is the type of interindividual

variability then that contributes to the

variability in drug safety and and

efficacy

another form of interindividual

variability that is important to

understand

is that involving drug drug interactions

i in this case here im illustrating it

with the the major p0 sip a

an enzyme that is very sensitive to drug

drug interactions both induction by

agents such as rifampin as well as

inhibition by agents such as

ketoconazole

the data that im illustrating here is

with the drug symbol statin a a a drug

used to to lower um blood cholesterol

levels

it is a sensitive a substrate in the

sense that that there are profound

changes in its systemic exposure

following coadministration of both

inducers of the predominant enzyme that

catalyzes it elimination a as well as

inhibitors and looking then at the

concentration time profile for

simvastatin

under control conditions here the

circles dark circles

you can see the effects of

coadministration with both rifampin

shown down here as well as the inhibitor

thinking first about induction what you

see is coadministration of rifampin

leads to over 90 reduction in the

systemic concentration of simvastatin

that major reduction in systemic

exposure basically leads to a loss of

efficacy

in contrast coadministration with an

inhibitor of ca leads to a marked

increase in the systemic concentrations

of simvastatin resulting in increased

risk for adverse side effects such as

myopathies

that profound change in the disposition

of simvastatin is a consequence of the

fact that it is on the interactions can

occur both in the intestine as well as

in the liver and both

interactions there influence the

availability of that sensitive substrate

simvastatin

following oral administration and that

is a consequence of the fact that

simvastatin has a very high intrinsic

clearance catalyzed by ca enzymes

not all ca enzymes um

exhibit this high uh intrinsic clearance

and high first pass elimination in the

gut and the liver and thats depicted by

looking at the effects of inhibition by

ketoconazole on three benzodiazepines

midazolam

[Music]

moderate extraction ratio drug it has a

relatively high intrinsic clearance and

there is is first pass elimination in

both the liver as well as the the

gastrointestinal mucosa in contrast

alprazolam has a low extraction rate

ratio um

in in both the gut as well as the liver

a low intrinsic clearance but its

elimination is is controlled by the same

enzyme as midazolam

a and therefore the effects of

ketoconazole are different you get a

course for both substrates of a an

increase in the auc following

coadministration with ketoconazole for

both midazolam as well as al presley

but what is different is the effect on

first pass elimination in the case of

midazolam ketoconazole can basically

reduce the extraction ratio both in the

liver as well as the gut and lead to an

increase in the bioavailability of

medusa from 0

up to almost 00

and that results in not only the the

extension of the halflife of the drug

as a result of the reduction in systemic

clearance but a profound increase in the

cmax as a result of the increase in the

bioavailability of midazola and

therefore if you look at the area under

the curve uh with with coadministration

of

midazolam and ketoconazole compared to

the control you see fold changes in

that ratio in contrast for midazolam

its a very modest one and a half fold

increase in the auc following key to

console administration so thats an

important um

observation

and basically it illustrates the

importance of understanding the the

sensitivity of a particular drug to

biotransformation by sip a as well as

is it the dominant route of elimination

another um

aspect of of variability that can be

found within the population is

variability that is a con consequence of

age

age in fact has an impact on the

abundance as well as the activity of the

drug metabolizing enzymes illustrated

here and looking at the cytochrome p0

activity the most profound changes

happen early in life

most of the hepatic drug metabolizing

enzymes are actually turned off in the

fetus during fetal development

and theyre triggered and turned on at

birth probably by epigenetic phenomena

however the time course of expression

varies from form to form and as a

function of age and this variability

this age dependent change in in p0

activity is referred to as the ontogeny

that is the ontogenic development of

this trait here relative to the adult

trait here

and so this is data that was part of a

metaanalysis of diagnostic substrates

for the different p0 forms

that have been studied in the pediatric

population and looking at the fraction

of that activity as a function of the

adult and shown here then is is the for

the individual forms the fraction of

activity starting from very low to

negligible at birth increasing for some

forms very very rapidly for other forms

more slowly

and even very slowly in the case of

sipe here so

differences in the time course driven by

presumably differences in the regulation

of the hepatic p0

as a function of age contribute to this

ontogenic if you will profile for the

different p0 forms and of course this

is a very important from therapeutic

perspective of understanding this time

course when thinking about how to dose

drugs that are

predominantly eliminated by one p0

form or another

another important source of variability

in drug metabolizing activity that

impinges on both drug safety and

efficacy are the changes that can occur

in pregnancy

um pregnancy is is a very profound um

state in a woman

involving men of course many changes

that are that are are

mediated for

the development of the fetus but there

are also changes that occur that

influence the disposition of a drug a

few of them are illustrated here changes

that can affect gastric emptying

cardiac output the extracellular fluid

space that drug can distribute into even

the fat compartment the drug

distributed to

for drugstore obviously its the changes

that happen in the kidney that are

important influencing renal clearance

and then in the context of this talk

here its the changes in p0 um as well

as ugt activity that can occur in the

lim in the liver during pregnancy

and thats illustrated here with with

the substrate a diagnostic substrate of

cypa

midazolam

studies that have been conducted

typically because pregnancy is is

unpredictable by comparing the

disposition of a probe drug like

midazolam during pregnancy illustrated

here in in this concentration time

profile and comparing it to the

postpartum period where the baby has has
been delivered and then presumably the
womans liver
function and regulation is returning to
a baseline and whats shown here is the
increase in midazolam that occurs uh
following parturation and and
with lower aec following a fixed dose of
midazolam
during the pregnancy period this of
course then implies that theres an
increase in the the
midazolam oral clearance or cl over the
bioavailability of midazolam and that
has that increase has been attributed to
an up regulation of pa
by hormones
released during pregnancy in particular
progesterone as well as potentially
placental derived fetal growth hormone
there are many other changes that occur
for other p0s during pregnancy um that
that are also as profound and there are
some p0 activities that are actually
decreased during during pregnancy
finally just a few things about um

other

sources of interindividual variability

um during drug therapy of course one

needs to think about the function of the

liver itself

and the the impact that liver disease

can have on those metabolic activities

as

healthy functional tissue in the liver

is replaced as a consequence of of

disease

in particular during the most severe

stages of cirrhosis you generally will

see a reduction in the abundance of

liver enzymes as well as their their

activity their intrinsic activity and

this leads then to reduced ability to

eliminate drugs by the liver there are

also reductions in the functional blood

flow to those those um the the healthy

hepatocytes that remain in liver disease

and that also contributes to a reduction

in clearance other changes that can

influence the disposition include

changes in protein bite

the disposition of both oral and as well

as perennial or iv

doses of both low and high extraction

drugs are going to be influenced by

liver disease

of course

the the degree to which those activities

are affected is a consequence of the

severity of the of the liver disease

itself depicted here is is is basically

the clearance catalyzed by

different cytochrome p0s towards probe

substrate so those enzymes

with increasing severity of liver

dysfunction to the point of either

hepatic decomposition or the most severe

hepatic renal syndrome which will lead

to death

and what you can see here is that all

p0s decline as a consequence of these

decrease in hepatic function but they do

so at a different rate sip c9 seems to

be the most sensitive to early changes

in hepatic function with mild liver

disease

whereas others like sip to e

are basically somewhat resistant and

only decline in function with the very

very end stages of liver liver disease

others fall in between to varying

degrees and so its important then to

understand

that the the change in in the the

metabolic activity that is going to

occur as a consequence of of decreased

liver function but also that the time

course is going to vary depending on

which enzyme youre is is catalyzing the

clearance of a particular substrate

and so and finally then summarizing this

introduction to phase one metabolism

first that that phase one metabolism is

the predominant route of drug clearance

um and and first pass elimination

contributing to the elimination of a

majority of the drugs that are are on

the therapeutic

are on the market today for the

treatment of disease

those enzymes are localized primarily in

the liver and the small intestine

both contributing to first pass

elimination and of course the liver

contributing predominantly to the the

elimination following um

a drug in the systemic circulation

the p0s are the dominant uh phase one

enzymes um theyre multiple forms of

cytochrome p0 livestock elimination

they have um in significant uh

differences in abundance both in the

liver and the intestine and thats a

consequence of both genetic factors as

well as um

age sex and

transformations during life cycle and

then of course disease

lastly there are a variety of exogenous

sources of interindividual variability

that can affect both the abundance of of

a drug metabolizing enzyme and its

intrinsic activity

um in particular enzyme inhibitors and

inducers with sip a being the most

sensitive of the cytochrome p0s to

these effects because of the of the the

fact that is expressed in both the liver

and the small intestine and for

sensitive substrates can contribute to

extensive first pest metabolism but

there are a lot of other factors that

contribute to that variability even

things as simple as light light uh dark

cycles

and and diurnal variation even solar

cycles that influence um the abundance

of molecules such as vitamin d that

regulates um cytochrome p0s like a

and lastly

a not well understood

domain of epigenetic changes that happen

as a consequence of the life cycle as

well as

the effects of

foreign molecules

and disease states on

the expression of the the drug

metabolizing enzymes

and so with that i want to conclude this

presentation and introduction to

the phase one metabolism of drugs and

xenobiotics

thank you