we are honored to have dr kathy

dr giacomini is professor in the

department of bioengineering and

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in addition dr chacamini is the

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she received her phd in pharmaceutical

science from the state university of new

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postdoctoral fellowship training at

stanford university

dr giacomini is considered a leader in

the field of membrane transporters she

is a member of the national academy of

medicine

please enjoy the presentation today

hello im kathy jacomini im a professor

at university of california san

francisco

my research area is in membrane

transporters and today im going to talk

to you about membrane transporters in

clinical pharmacology and im going to

emphasize the aspects of membrane

transporters that i feel practicing

clinical pharmacologists need to know

about

so

ive divided my talk into three major

sections

first im going to be talking about

basic transporter biology and ill give

a brief overview here in that area and

that will be followed by two aspects of

membrane transporters that are important

to clinical pharmacologists the first is

transporters as mediators of drug drug

interactions which is very important for

drug safety and the second will be

transporter polymorphisms or

pharmacogenomic studies

which are relevant to membrane

transporters and to clinical

pharmacology so let me begin with

basic transporter biology

um all right i begin each one of these

sections with key points and i will end

on the same key points so the key points

that im interested in making here and

that i feel is important for you as

future clinical pharmacologists

is first of all transporters are

important in drug disposition and

response and those are generally in two

major super families the solute carrier

superfamily slc and the atp binding

cassette super family abc

second point is transporters are

integral membrane proteins with multiple

transmembrane domains which which

facilitate membrane passage of their

substrates

and the third point is transporters may

be primary active

secondarily active or facilitated and

their kinetics follow michaelismenten

equations

okay so this slide reminds us that

transporters are expressed on cellular

membranes and im just reminding you

that there are many cellular membranes

in within one cell so there are

mitochondrial membranes lysosomal

membranes membranes in the golgi or

endoplasmic reticulum and each one of

these subcellular organelles have

membrane transporters which shuttle

compounds in and out of the organelle

but the most important place

that transverses are expressed for

clinical pharmacologists is on the

plasma membrane and im showing you that

right here and all of the transporters

that ill be talking about today are

plasma membrane transporters

this slide is to remind me of and remind

you that

the plasma membrane and indeed all

cellular membranes are hydrophobic and

as such they control access to the

intracellular environment

and molecules really have to

get across that plasma membrane and few

of them can without the aid of some kind

of a protein and those proteins are

usually our term transporter proteins

okay so transporters are are needed for

hydrophilic substrates to cross

biomembranes um and for molecules which

are hydrophobic they can cross

biological membranes by simple diffusion

and you can see that process right here

and these molecules are hydrophobic they

do not require a protein to traverse the

plasma membrane however many other

molecules especially hydrophilic

molecules do require

membrane proteins and transporters per

se to cross the plaza membrane and im

showing you here different types of

transporters which ill describe in

detail im in the subsequent slides

but i want to remind you that anything

that is transported via a transporter is

called facilitated transport and

facilitated transport

which is facilitated if you will by a

transporter can be divided into two

major class categories one is

facilitated diffusion facilitated

diffusion doesnt require energy it

moves substrates

in and out actually of a cell

across the plasma membrane in accordance

with the electrochemical gradient

the second process um is called active

transport active transport requires

energy and may move substance substrates

against a concentration gradient for so

from low concentration to high

concentration and ill describe

facilitated transporters and active

transporters in the next few slides

so here im showing you three different

transport mechanisms

a uniport an antiport and a symport and

lets begin first with a uniport

transport mechanism a uniport transport

mechanism is a simple way that theres a

transport protein in the cell membrane

and its traverse and the molecules the

substrates shown here in orange are

traversing down the concentration

gradient and the tren and the pl and the

transporter is being used if you will to

facilitate that crossing of that

hydrophobic molecule which is not going

to cross

the lipid bilayer without this transport

protein so one molecule the substrate

moving through is a uniport mechanism

and then there are two other uh

processes that im showing you here are

transport mechanisms symporters and

antiporters let me describe an

antiporter first so an antiport

mechanism the substrate shown here is

moving

here

maybe downhill or maybe uphill

in in accordance with the

electrochemical gradient but its

exchanging with um with another counter

ion or an ion and that purple is that

iron that ion may be

a proton gradient that ion may be some a

chloride

molecule some kind of an of a solute is

moving in exchange for the movement of

the substrate and so its an exchange

mechanism

um a very wellknown exchanger that

works in this way that we will be

discussing later on is

multidrug and toxin extrusion protein

mate

the third mechanism that im showing on

this slide is a symporter symporters are

quite important and theres a very

wellknown symporter the sodium

the serotonin

transporter which is a sodium serotonin

symport mechanism so here would be

serotonin moving across the plasma

membrane into the cell and over here

is

maybe the sodium molecule moving also

together with um

with serotonin actually driving

the

the uphill movement of serotonin

from from maybe a lower concentration to

a higher concentration

transporters exhibit characteristic

kinetic properties

they are saturable

um they are inhibitable and they are

temperature dependent so saturable is a

property that were all aware of with

the transport mechanism that is the

transport mechanism will increase as

concentration increases and then a

maximum rate

will be achieved and that maximum rate

will depend on

a couple of things one is the number of

transporters in the plasma membrane

secondly transporters are inhibitable

that is another substrate or a socalled

inhibitor may inhibit the transport of a

test substrate

so thats the second property that

characterizes a transporter and third is

terran sporters are very temperature

dependent that is at low temperatures

they work very poorly they have an

optimal temperature its usually

degrees

which is you know our biological

temperature

um so lets examine that property of

saturability um and so what im showing

you here is a transport kinetic

properties of two molecules one molecule

is going by simple diffusion

and the other by a carriermediated

process or a transporter

um

the simple diffusion the molecule thats

diffusing going by simple diffusion

were plotting here solute concentration

so this is increasing concentration

and were plotting on the yaxis rate of

transport so this is increasing rate of

transport and you can see a molecule

that

crosses the biological membrane by

simple diffusion its rate of transport

is just plain linear as concentration

increases the rate of transport

increases however a carriermediated

process or a transportermediated

process will also do the same at lower

concentrations that is as concentration

increases the rate of transport will

increase and then it will saturate

and you will get to a maximum transport

rate the socalled vmax

and so thats one of the properties of

of a

transporter is that there will be a vmax

that vmax is dependent on the

transporter and the particular substrate

thats being transported

the other uh kinetic parameter that

characterizes this transport process is

a km

so this slide what im showing you here

now is the equation

for something

for a compound which diffuses so rate of

diffusion is simply directly

proportional to the concentration of the

molecule which is diffusion diffusing

and that proportionality constant is

often called a diffusion rate constant

kd

for the saturable process

now we have the typical michaelismenten

equation where the rate of transport at

low concentrations is linear

but its characterized by a vmax when

you get to the higher concentrations

its also characterized by a km and as

you may remember the km

is the concentration of the solute or

the substrate which is being transported

at half the maximum

velocity or transport rate so you find

half the vmax right here and you look

for that concentration and that

concentration is the km so this

michaelismenten equation which

characterizes many enzymatic processes

that im sure everyone here is familiar

with

also characterizes transport processes

sometimes vmax for transporters rather

than being termed

maximum velocity is termed tmax

transport maximum

but its the same thing it means the

maximum rate of transport and as i said

its characteristic of the transporter

and the substrate that its transporting

now im going to describe

the major transporters

and the major transporter families that

we have to be interested in as clinical

pharmacologists or aware of the first is

the solute carrier super family and im

showing you here

the super family which is clustered

according to homology relationships and

there are 00 transporters here

clustered according to homology

relationships into over 0 distinct

families

so theres a wealth of solute carrier

transporters in the human genome this is

only in the human genome if we were to

go to other species thered be even more

even all the way down to very simple

single cell organisms transporters

characterize very simple single cell

organisms like bacteria

these solute carrier superfamily

transporters are all facilitated

transporters in one way or the other

they may be uniporters as i showed you

on the previous slide they may be

antiporters or symporters

so they may move substance their

substrates against a concentration

gradient

in accordance with a gradient of for

example a sodium ion gradient like the

serotonin transporter but they

do not rely directly on atp hydrolysis

so they are not atp binding

cassette family members they are

distinct from from that major

superfamily which ill describe in a

minute now as clinical pharmacologists

you have to keep your eye on many of

these transporters because many of them

will be drug targets

and many of them may play a role in drug

disposition but i have today

focus very clearly

this lecture on transporters that play

an important role in many different

drugs so we wont have to think about

every one of these

00 or so transporters in the slc

super family notable slc transporters

that i will be talking about today are

transporters in the slc family or

organic ion transporters um and slca

is organic cation transporter one or

encodes organic cation transporter one

in fact all the transporters by the way

in the slc super family have an slc

designation for their gene name and they

may have a familiar name like organic

cation transporter one for the protein

name so

oct

and they also have an abbreviation like

oct the protein name often has an

abbreviation so

slca is the gene organic cation

transporter

is the name of the protein that is

encoded by the gene and oct is the

familiar name that youll youll be

familiar with when you read the

literature um and then im also showing

you organic cation transporter two and

the difference between these two

transporters is theyre encoded by two

distinct genes one is in the liver this

one

and one is in the kidney this one

and theyre also expressed in other

organs in the body or other tissues in

the body but theyre largely expressed

in these two organs and as you can

imagine play a role in hepatic

disposition of drugs and renal

disposition of drugs so theyre very

important for drug elimination

um ano another notable slc

transporter

family member that im going to describe

today that clinical pharmacologists

should know about is

oatpb that is the familiar protein

name of this transporter which is

encoded by the gene

slc0b

and that is an organic anion

transporting polypeptide b its found

in the liver well describe it a little

bit later on it brings a whole lot of

drugs into the liver where they can be

metabolized or interact with their

biological receptors and right on the

same locus in the same chromosome and

right adjacent to slcob is

slcob and that is also expressed that

that that gene is also expressed on the

plasma membrane on the sinusoidal

membrane of the liver and it encodes

oatpb also a very important

transporter protein that ill mention a

little bit later

all right so those are the notable slc

transporters and remember that slc

transporters are facilitated

transporters they do not rely directly

on atp hydrolysis they may be coupled to

an ion gradient or they may function as

uniport transport mechanisms

now im going to go to the other super

family and that is the atp binding

cassette superfamily and this is also

important for

drug disposition and response

transporters in this particular super

family here instead of you know

0 different families with 00 different

uh transporters there are seven

different families and they in the human

genome and they encode some different

transporters

now

the transporters the super family the

families within the super family that

are important and that we will be

describing today our abc b family the

abcc family and the abc g family and

ill talk a little bit about

transporters in these three different

families but the main thing to note and

im showing you here

a molecule an atp binding

a cartoon representation of an abc

transporter and this is intracellular

and this is extracellular well

as the name suggests atp binding

cassette or abc

transporter family these transporters in

contrast to the slcs rely on atp

hydrolysis

and they will directly pump uh their

substrates um against a concentration

gradient and directly using the energy

supplied by

atp

um

they are all the ones in the in the

human genome that we will just that we

will talk about today are efflux pumps

and so we have to think about them

whereas the slc transporters move their

substrates in accordance with the

electrochemical gradient or

or the ion gradient that theyre coupled

with

abc transporters are effluxing their

substrates and well talk about that in

the next slides

so

the notable abc transporters that you

will learn a lot about um

as

are abc b family members and the most

important one in fact probably the most

well known transporter in the human

genome is abcb that is the gename the

protein name just like the slcs theres

a protein name is p glycoprotein

and the abbreviation is pgp

and theres another abc trans b family

member abcb which is bcep the bile

salt export pump i wont be describing

this transporter much in this particular

lecture but i will say a word or two

about pgp

and then the abcc family members that

are very important in clinical

pharmacology are abcc and abcc these

transporters are in the liver um they

may be in other organs as well the

kidney for example but theyre notable

in the liver um and sometimes in the

kidney and theyre multidrug resistance

proteins

and they are mrp and mrp and i

probably wont talk too much about these

two transporters just know that they

play a role in multiple drugs

and they play a role in their hepatic

and renal disposition

and the final protein that i will be

spending a little bit of time on today

is abcg family member abc g incl

encoding a protein called the breast

cancer resistance protein or bcrp

and bcrp was discovered as its name

would imply as an efflux pump in breast

cancer

cells which pump chemotherapeutic agents

out of the cells

and therefore confer resistance

of the breast cancer to chemotherapeutic

agents so

im not going to describe that part of

the role today but i will talk about the

more endogenous role of this transporter

playing a role in drug disposition and

response

in pain in many patients

so let me bring up my key points and

just rereview them again in basic

transporter biology so number one

transporters which are important in drug

disposition in response are generally

found in two major super families the

solute carrier super family slc and the

atp binding cassette super family abc

the solute carrier super family slc

remember is a facilitated encodes and

facilitated

transporters which may be secondary

active or maybe simple

facilitated diffusion transport

transporters

the abc

superfamily

is comprised of transporters which rely

on directly rely on hydrolysis of atp

pump their substrates against a

concentration gradient and our efflux

pumps in the human

genome transporters are integral

membrane proteins with multiple

transmembrane domains they facilitate

membrane passage of their substrates in

general the substrates are hydrophilic

and would not diffuse but they may also

interact with hydrophobic substrates

thirdly transporters may be primary

active

and that primary active means it they

directly rely on hydrolysis of atp for

their energy source secondarily active

which means they rely on coupling to an

ion gradient like a symporter or an

antiporter or just plain simple

facilitated transport mechanisms which

are the uniporters that i described in

an earlier slide they follow

michaelismenten equations their kinetic

properties follow michaelismenten

equations which basically means that

they will exhibit the property of

saturability when you get to high

concentrations they will go at a va at a

maximum transport rate

and they will also exhibit the property

of um

of uh

of a they will also have it be

characterized by a km a km being the

concentration of the substrate at half

the maximal transport rate so those are

my key points in the basic transporter

biology

part of this lecture

weve concluded the basic transporter

biology overview section and im now

going to turn to clinical pharmacology

and transporters that are important for

clinical pharmacologists and the first

topic that ive selected is transporters

as mediators of drug drug interactions

and then ill segue from there to

transporter polymorphism so lets start

with transporters as mediators of drug

drug interactions

ive listed here some key references for

you

and one of them the top one in nature

reviews drug discovery i suggest

everyone reads because that really is

that really

ushered in the whole area of

transporters in drug drug interactions

and it was published in 00 in nature

reviews drug discovery by the

international transporter consortium and

im showing you their logo up here

and then ive listed two other

references

on transportormediated drugdrug

interactions

now

as with the other section i have some

key points that id like to make in this

particular section one is that there are

key transporters that are potential

mediators of drug drug interactions its

not all the transporters i showed

for example all the 00 slc transporters

that i showed in one of my earlier

slides its really focused on these

particular transporters that im listing

here pgp

which we described earlier an abc

transporter bcrp also in abc

transporters and then oc mate and

mate k

those are in the kidney 00 and 0 in

the kidney and oatpb and b in the

liver so we will be discussing this for

my key points

second key point there are decision

trees

which the fda has created which will

inform clinical pharmacologists

on uh on whether to con conduct a

transport or mediated drug drug

interaction

study clinical study and those decision

trees have been described they are well

published and well known and im going

to describe how you would use a decision

tree to help inform you on whether you

want to do a clinical drug drug

interaction study

the third

key point is there are two major types

of decision trees substratebased and

inhibitorbased and i will describe

those as well

and finally inhibitor decision trees

require a knowledge of the drug

concentrations in vivo and the in vitro

inhibitory constants and ill describe

that briefly

so let me begin this part of the lecture

so first of all lets go back to the

early 000s

and and i dont know some of you may

remember but a

very wellknown statin was withdrawn

from the market because of fatalities

and that was cerivastatin it was

withdrawn from the market in 00

because rhabdomyolysis

a lifethreatening side effect to this

particular state and actually to many

statins although rare for the other ones

but more common for three vostatin uh

had had occurred in a number of patients

and i think there were like

fatalities

which were attributed to cereba statin

because of rhabdomyolysis so it was

withdrawn from the market

and at that point in time they recognize

that a drug drug interaction may have

been the cause of the withdrawal and

ill describe that

um so

they noted that patients

who were also on cereva statin and

gemfibrizol

those patients were at a higher risk for

rhabdomyolysis

and so when they went back and examined

this or when the scientific community

went back and examined what was the

cause of this it turned out that there

was a drug drug interaction but it was

not mediated by an enzyme prior to that

date all drug drug interactions had been

thought to be mediated by drug

metabolizing enzymes certainly important

ones

important

it was mediated in part by a transporter

so im showing you here

sort of a historical study where youre

looking at cerevastatin plasma levels in

the lower curve here

in a group of uh of patients probably

healthy volunteers here um and then

cereba statin plasma levels when it was

administered concomitantly with

gemfibrizol

and you can see the levels are four to

five times higher

so gemfibrizol has caused a drug drug

interaction and the plasma levels of

cerevastatin are much higher in patients

who would be on gemfibrizol together

with cereba statin and that may have

been why patients who were on jim

fibrazole

were at higher risk for cerevastatin

induced rhabdomyolysis

and when the mechanism

was delineated it turns out that it was

that this interaction

was mediated by

oatpb that hepatic transporter that i

highlighted earlier and then ill

describe in a little more detail in a

subsequent slide and also by an enzyme

sipc but its oatpb seem to be the

major

site for this drug drug interaction so

what happened is

cerevastatin was going into the

hepatocyte ill just call it cerive its

going into the hepatocyte where its

metabolized by cypc

however when gemfibrizol

or jibfibrizol was on board

it inhibited

oatpb so cerevastatin could not enter

the hepatocyte it was blocked and its

levels went up and thats what youre

seeing here

those higher levels and so it became

recognized in 00 that transporters

play a critical role in drug drug

interactions and may be very important

in the safe use of medications and so

what happened at that time was there was

an explosion in the world of which

transporters which ones are causing this

besides oatpb

and thats when the international

transporter consortium

was formed

and what we did and i was one of the

people who played a role in forming the

international transporter consortium is

we began to

curate the literature and we published

this paper that i highlighted earlier in

nature reviews drug discovery in 00

and we said it wasnt all those

transporters that people had to worry

about there were just a few that were

very important in mediating drug drug

interactions and those were the ones

that people drug developers had to think

about and clinical pharmacologists had

to think about

in terms of both their research and

patient practice

and so how did we identify those

transporters we looked in the literature

uh for transporters that had a high

level of evidence that they played a

role in drug disposition and there had

to be in vitro evidence

all right that here

in vitro so that means in a cellbased

assay we had to show that the

transporter was mediating the transport

of drugs and then we looked for clinical

evidence that is there was some clinical

evidence that that transporter was

causing or was responsible in some way

for a drug drug interaction so there had
to be several lines of evidence here and
we also looked at genetic polymorphisms
but ill save that until the last part
of my talk
after that paper was published two years
later the fda published a guidance for
industry and it was on drug drug
interactions and in the guidance were
decision trees and ill be describing
decision trees those same decision trees
well not the identical ones but similar
decision trees had been published in the
earlier paper by the international
transporter consortium which by the way
is a consortium of academic scientists
industry scientists and fda scientists
so it really is a very good consortium
to really look over the literature and
describe what are the important
transporters
for that mediate drug drug interactions
and from the itc and im going to
unhighlight that so you can see from
the international transporter consortium
recommendations these transporters

appeared as being most important and

ill describe those on the next slides

so lets start with the intestine so

what youre looking at over here is you

know a representation of the intestine

some artist rendition of the intestine

and im reminding you of an intestinal

epithelial cell here is the intestinal

lumen over here

and over here is the blood side so when

you take a drug it starts out in the

lumen

and then it may be absorbed and then it

crosses into the blood this way

crosses that intestinal epithelial

membrane into the portal circulation

during the drug absorption process

in the intestine two major transporters

were called out by the itc and

subsequently in the fda guidance

one is p glycoprotein and the second is

bcrp as you may recall both of those are

abc transporters they rely on atp

hydrolysis they efflux their substrate

so what you can imagine these two

transporters are doing is they are

preventing drug absorption right because

theyre keeping the drug in the

intestine intestinal lumen um

but they can mediate drug drug

interactions

and if one were to inhibit one of these

then it couldnt efflux a drug and more

drug may be absorbed

and so

well describe that

later so those were the two that were

called out in the intestine im showing

you in the smaller font other

transmitters are there just to remind

you that there are a host of

transporters that do play a role in drug

absorption but these werent called out

as the most important transporters that

clinical pharmacologists and drug

developers need to be aware of

um

this

cartoon and let me erase the intestinal

piece

shows now the transporters that were

highlighted that played a role in

hepatic drug disposition

and so heres the liver and what youre

looking at here is the sinusoidal

membrane

so that faces the blood

and then

this is in the portal circulation the

portal blood and the hepatic arteries

they enter the liver and uh the drug

will be present in the in the sinusoids

and may enter the hepatocyte where the

drugs may be metabolized over here we

have the canalicular membrane

and the canalicular membrane lines the

bile doctor goes into the bile duct and

there are transporters there which may

move drugs from the hepatocyte into the

bile and two transporters were called

out as very important on that membrane

bcrp

yet again its found in the intestine

on the luminal membrane or the apical

membrane its also on the canalicular

membrane in the hepatocyte and pgp

and then on the on the basal lateral

membrane or the sinusoidal membrane

oatpb and

oatpb those slc transporters mediate

influx and the one that i described in

my opening

remarks to this particular section

um

that mediate that mediated the

interaction between jim fibrezel and um

and cerevastatin oatpb

so those were called out as being very

important transporters that people

should be uh aware of oct organic

cation transporter was not in the

initial guidance of the fda they are in

the process of publishing a second

guidance and it may appear im not sure

this is an organic cation transporter so

it transports basic drugs unlike oatpb

which is mostly transporting

acidic drugs and it also plays a role in

drug disposition but has yet to be

called out by the international

transporter consortium or the fda

um

now moving on

to the kidney

several transporters play a role in

renal drug elimination so here again

youre looking at a proximal tubule

youre looking at a cell and this is a

proximal tubule cell and over here is

the lumen or the urine side

and over here is the blood side and so

drugs which are actively secreted have

to traverse both membranes to get into

the urine

and the transporters that play a role in

basic drug renal secretion are octu

on this membrane bringing drugs from the

blood into the tubule cell and on this

membrane mate and mate ive just

shown it here

and they bring basic drugs into the

tubule limit

for anionic drugs or acidic molecules

organic anion transporter one and three

one and o move the drugs from the blood

into the

proximal tubule cell and then those

drugs may move out by a variety of

different transporters maybe pgp bcrp

and some other transporters here so the

transporters that have been called out

that our sites for drug drug

interactions are pgp bcrp in the

intestine and over here in the liver and

oatpb oatpb

and then in the kidney oc oat one oat

three

and eight one and two and and again pgp

and bcrp play a role there

i wanna highlight for you um

that there are many transporters sort of

databases and one of them is here at

ucsf its ucsf fda transportal and it

lists a number of in vitro and in vivo

potential

drug drug interactions and and data

that have to do with with drugs

transporters uh maybe michaelismenten

parameters for in vitro for in vivo it

really describes the database will

describe the

clinical drug drug interaction and also

give you references that you can link to

in pubmed so there are there are

databases that you can look up

transportermediated drugdrug

interaction studies either in vitro or

in vivo get information from those

databases and identify

references but those tend to be for more

historical drugs for newer drug

molecules the database is not yet is not

being updated at this point in time

id like to now turn to the decision

trees that ive been talking about

so youre developing a drug so clinical

pharmacologists just as youre all aware

play a role in therapeutics they play a

role in academic institutions but

theyre also found in industry and in

industry clinical pharmacologists need

to be aware of

when to conduct a clinical drug drug

interaction study and one thats

transporter mediated and so here the fda

and ive taken this from the fda 0

guidance

set up a decision set up some decision

trees

and im showing you here the decision

tree sort of an overview of decision

trees for substrates not inhibitors so

you have a drug which may be a substrate

of one of these transporters could it be

involved in a drug or a victim if you

will of a drug drug interaction

and the decision trees are

divided into

three major categories pgp and bcrp

oatpb and b and then renal

transporters the oats the oc and mate

im only going to describe one

aspect of the decision tree im only

going to focus today on pgp um im

really on pgp although the same

principles will apply for bcrp ive

listed here the url

where you can find these guidances but

really you can simply google

fda guidance drug drug interactions and

youll find the various guidances and

the updated guidances

that are being published by the fda

so let me start with pgp

so reminding you

that um pgp sits

on the luminal membrane or the apical

membrane of the intestinal epithelium

its effluxing its substrate so its

really preventing

drug absorption if you will or sort of

limiting drug absorption so its

substrates are moving in this direction

here from if you will the blood side

back into the intestinal lumen and if

you have a particularly good pgp

substrate pgp will keep that drug from

being absorbed and many drugs that are

pgp

substrates may have bioavailabilities

around you know 0 0 percent low

bioavailabilities depending upon

the degree of interaction of the drug

with pgp

so you can imagine a drug drug

interaction here would be very important

because if you had an inhibitor of pgp

a drug which inhibited pgp and you were

developing a substrate that inhibitor

could actually cause your drug to be

absorbed more because it would not be

effluxed and so youd get more

absorption youd get higher drug levels

increase sea levels the drug

concentration levels and potential

toxicities

so

very important to understand whether a

new drug in development is a pgp

substrate

so the way thats done is an in vitro

study and remember these guidances

really

are instructing people to conduct in

vitro studies and use that in those in

vitro studies and perhaps some modeling

to inform whether or not you want to do

a clinical study and what that clinical

study should look like a little bit

so the in vitro system for studying pgp

is a transwell system and what a

transwell system is its a well within a

well and so this is the first well here

shown in this uh more royal blue and

then in the turquoise blue is the within

the well the well uh the outside well

and right here on this membrane is a

permeable membrane

and you seed your cells and your cells

may be calco cells or intestinal

epithelial cells that are over

expressing pgp

but theyre polarized cells and their

apical membrane uh is facing upward just

like over here

this is the apical membrane here

and you put your drug your new substrate

you put your drug

into the inner compartment here and you

measure the flux from compartment a this

is called compartment a the apical

compartment to compartment b the basal

compartment so you measure this flux

from a to b

now

that experiment is done you do a

subsequent experiment

where you put the drug on this side over

here on the basal compartment and you

measure the flux in the other direction

b to a so you have an a to b flux and a

b to a flux if there were absolutely no

transporter involved a to b should be

equal to b to a the flux should go

at the same rate in in both directions

however if theres an efflux pump what

do you think would happen the b to a

flux is going to go in a greater rate

than the a to b flux

so you take a flux ratio b to a

divided by a to b

and if that flux ratio

is greater than

maybe its a pgp substrate right because

its being fluxed into the apical side

at a much greater rate or at least

twofold greater than its being fluxed

into the basal compartment

so

after youve determined that flux ratio

if the flux ratio is greater than two

you may say to yourself hmm this looks

like a pgp substrate but you have to do

another experiment because there may be

other abc transporters in that membrane

maybe you know transporters you hadnt

thought about because you know the cell

membrane its a cell membrane and it has

other transporters so you put in a pgp

inhibitor and one such inhibitor may be

verapamil um that that but there are

other inhibitors that you could use um

so you put in varapamil rifampicin a pgp

substrate or an inhibitor but one that

will inhibit and what should that

inhibitor do well that should reduce b

to a flux because youre inhibiting pgp

influx so a to b to b to a flux might go

back to one you know because youre in

youre youre

reducing the

um

the flux the active transport flux by

pgp into the apical compartment

so you add that inhibitor and if the

inhibitor reduces the b to a flux or

changes the flux ratio from for

example or 0 whatever it is greater

than

back down to or you know just reduces

it it probably means the drug is a

substrate of pgp

so now you have some in vitro evidence

and let me take you to the decision tree

and lets just walk through it step by

step so the first thing you do is you

set up your bidirectional flux assay

and you ask whether the flux ratio is

greater than two so this is step one

and you get a decision its a decision

tree so you get yes or no so lets say

no its less than two end of story it is

not a pgp substrate or its a poor pgp

substrate and you dont have to be

concerned with a clinical drug drug

interaction study

however if the ratio is greater than two

now

youre a little bit aware that this

that this is could be potentially

important so you add an inhibitor

if the inhibitor doesnt inhibit the

flux ratio and it stays at or 0 or

whatever it stays at something its not

inhibited then other transporters not

pgp are responsible for that greater b

to a flux than a to b flux

if however so again youre going to have

to now figure out what those other

transporters may be

however if the flux ratio is greater

than

its inhibited by one or more pgp

substrates

then you can conclude here in the green

yes its probably a pgp substrate pgp

may be playing an important role in its

absorption and maybe limiting its

bioavailability there may be a need for

a drug drug interaction study and there

the fda gives some guidances as to what

you might consider what because your

drug is a substrate right and so now you

want to

administer your drug with an inhibitor

of pgp and see if your drug levels go up

and so they might suggest give it with

amiodarone ketoconazole cyclosporine

itll depend upon the therapeutic class

of your molecule

and other considerations um which you

can plan and together

work out a plan for a drug drug

interaction

study so this gives you an idea of a

decision tree where an in vitro

information which are much less

expensive and much

quicker to conduct

can provide information on whether or

not to conduct a clinical drug drug

interaction study the fda has similar

decision trees for inhibitors instead of

your drug being a substrate of pgp what

if its not really a substrate but it

inhibits pgp now its going to

perpetrate drug drug interactions wont

be a victim of a drug drug interaction

but itll cause interactions with other

drugs and that could be an important

concern

so there are inhibitor decision trees as

well that im not going to go through

but they will be very similar you just

walk down the in vitro methods are very

well described there are criteria and

those criteria trigger or not a clinical

drug drug interaction

study

so lets go back to the key points of

the drug drug interaction part of this

lecture

so first of all the transporters that

you have to be most concerned with that

are responsible and mediators of many

clinical drug drug interactions include

pgp

bcrp the abc transporters in the kidney

octu mate and mate k

four

drugs which are actively secreted and

these these transporters take up basic

drugs one and o

also in the kidney for acidic drugs

which are actively secreted and then

oatpb and oatpb

in the liver bringing a number of drugs

into the liver

where they can be

metabolized or interact with their

targets so key point one these are the

transporters you need to know about

key point two is there are decision

trees

and they will inform you using in vitro

studies

whether or not to conduct a transport or

mediated drug drug interaction and then

which transporter you need to focus on

and some information

from those studies about what drugs

you may need to use in your drug drug

interaction study there are two major

types of decision trees substratebased

and inhibitor based

we went over the substrate base

decision tree but you can go over the

inhibitorbased decision trees those are

very

clear and in the inhibitorbased

decision tree that will require a

knowledge of the kinetic properties in

vitro as well as the maximum

concentrations in the plasma in vivo

in the inhibitor decision tree

so that

concludes the second part on

of my lecture on clinical pharmacology

and transportermediated drugdrug

interactions

okay in this section im going to

describe transporter polymorphisms and

im going to talk to you about

pharmacogenomics

but instead of pharmacogenomics focused

on drug metabolizing enzymes im going

to talk to you about pharmacogenomics

that involve

polymorphisms in important drug

transporters

theres a key reference this was

authored by the international

transporter consortium and it was

published in i think 0

and it is focused on clinically

important transporter polymorphisms and

there have been a wealth of studies

published on transporter polymorphisms

and the role they may play in drug

toxicity drug response drug disposition

but this particular reference

really distills a lot of information in

the literature to the most important

transporter polymorphisms that we need

to be concerned with at this point

in time

so i have some key points for this

section as well first of all

polymorphisms in bcrp and oatpb play a

critical role in variation in drug

absorption disposition and response so

two transporters bcrp and oatpb and

im going to be talking about both of

them

secondly the itc recommends a strategic

approach to pharmacogenomic studies and

the strategic approach will involve

preclinical

drug clinical studies and you may do a

drug drug interaction study as part of

the polymorphism study

and then finally a pharmacogenomic study

if certain criteria are met so well go

through those

strategic approaches

as i go through this part of the lecture

so

in curating the literature and in

reviewing the literature the itc

used certain criteria to select what

they called these very important or

essential polymorphisms that people need

to be aware of in drug development and

in the practice of clinical pharmacology

and the three criteria that we used were

first of all we put a high bar that is

the transporter polymorphism had to be

significant at genomewide level

significance in a genomewide

association study and as you recall a

genomewide association study takes an
agnostic approach to discovering genetic
variants that underlie drug response
drug disposition and drug toxicity so
that was our first criteria its a very
high bar many transporter polymorphisms
have been studied in candidate gene
studies but they dont make the
genomewide
level significance
and im not saying that those are not
important those candidate gene studies
are indeed important but we decided that
we really wanted to focus on which were
the most important polymorphisms in
transporters
to study
the second criteria is we wanted them to
also be significant in multiple
candidate gene association studies so
not only in a genomewide association
study but in multiple candidate gene
association studies and finally that
there should be in vitro studies that
document that that transporter
polymorphism

actually exhibits functional changes

that its not simply in linkage

disequilibrium with the real

polymorphism which is causing uh the

variation in drug response but that it

itself is the culprit or causing the

variation in drug response so those are

the three criteria and as i go through

the polymorphisms that im about to

describe i will go through each one of

those criteria and show you the level of

evidence that we reviewed and that are

that is published in that particular

paper

so the first

polymorphism

transporter polymorphism

that uh met these criteria and their

only two

um

at that point in time there were only

two

um was slcob encoding oatpb

and it is and and the polymorphism is a

coding polymorphism and thats what this

c means

coding polymorphism

c

dot t to c so that means at position

um and that means a nucleotide position

in the cdna there is a polymorphism

in which certain individuals carry

instead of a t

have a c

and the rs number of the snip is shown

there remember all snips or single

nucleotide polymorphisms have these rs

numbers and those can be found in db

snip

that polymorphism in which the t was

changed to c

caused an amino acid change in the

protein so veiling at position

is changed alanine so individuals with

the t allele have the valine but the

individuals who have the polymorphism or

the c a level allele have the alanine

now im showing you here allele

frequencies of that polymorphism in

three different major ethnic groups in

the us

however the thousand genomes project has

the allele frequency of this

polymorphism in many more ethnic groups

so you can go to the refer to the

thousand genomes project and you can

find allele frequencies of this

polymorphism just look it up by gene

name and by rs number and you can find

the allele frequency in other ethnic

groups but one of the things youll note

and this is true for drug metabolizing

enzymes as well as transporter

polymorphisms is allele frequencies will

vary according to ethnicity um so

african americans have a allele

frequency of this c allele um whereas

europeans and asians have you know

allele frequencies the polymorphism is

associated with reduced activity and

ill show you those data

so thats the first one and ill

describe that in a little more detail

the second one that im going to be

describing today is abc g again this is

breast cancer resistance protein bcrp

here we again have a coding c

uh a c to a change so this is a

change to

from a c to an a theres the rs number

that also results

in an amino acid change glutamine to

lysine

qk

and as you can see the allele frequency

is very high in asian americans and

these are east asians at around 0

percent and lower in other ethnic groups

and that abc g variant also is

associated with reduced function

so let me start with oatpb or slcob

the valine alanine

so just reminding you here of heres the

transporter again on the hepatocyte on

the sinusoidal membrane and its in a

locus together with oatpb and the two

transporters work together and sometimes

very difficult they have very

overlapping substrate specificities

however the polymorphism is in the

coding region of oatpb so its oatpb

when we think about a genetic study

or a pharmacogenetic study that we have

to think about and its that particular

polymorphism in that gene so first of

all oatpb is a major hepatic

transporter for organic anions

or acidic drugs it transports statins

those are well known to be transported

by oatpb number of antidiabetic drugs

antiviral agents anticancer drugs so

it transports a variety of different

drugs or what you call xenobiotics so

its an important xenobiotic transporter

and both of these are located in one

particular locus slcob and b on

chromosome

now the first level of evidence was that

it had to be associated with

at genomewide level significance in a

genomewide association study so what

youre looking at here

is a typical manhattan plot and ill

just remind you of the essentials of a

manhattan plot what what is plotted is

the minus log pvalue

of significance of maybe 00 000 snips

and how significant they were in

patients with

statininduced myopathy and in this case

it was simvastatin and this paper was

published in 00 so it was among the

earlier genomewide association study

and you can see the mind the higher the

the higher the peak here or the higher

the data point

the higher the data point

the more significant because its a

minus log pvalue so this for example

variant here

the pvalue is 0 to the minus 9

times 0 to the minus 9 so definitely

reach genomewide level significance and

it turned out that that variant is

slcob the rs number i just gave you it

encodes that t to c change and that is

the valine alanine which well

describe and that came up in 00 as

being associated in a large clinical

study with statininduced myopathy at

genomewide level significance now you

can see it could have been anything was

associated with statininduced myopathy

but this variant really stun stood out

and thats been replicated in

additional studies after that uh

historical study in 00

okay

um whats very interesting about this

particular variant

is that individuals who are homozygous

remember you get one allele from your

father and one from your mother so

individuals that are homozygous for the

c or the polymorphic allele 0 percent

of patients

who have statininduced myopathy in this

particular study

were homozygous for the c allele thats

rather striking whereas if you carry the

t allele

or your heterozygotes

much fewer much lower percentages have

statin induced myopathy so care being

homozygous for this allele really

confers risk for statininduced myopathy

um and that that was shown very nicely

in this study so thats the first level

of evidence genomewide association

level evidence

the second level of evidence that we

look for is multiple candidate gene

studies and here is a basic clinical

pharmacology study so what youre

looking at on the left plot is

simvastatin

and simvastatin

in patients who are homozygous for that

c allele right here and these are

patients here and here who are either

heterozygous or homozygous for the t

allele so you can clearly see

individuals who are homozygous for the c

allele have much higher statin

levels on the same dose

and thats because theyre not bringing

the statin into the liver where the

statin can be metabolized

um and this has also been studied for

other drugs so the same allele and now

were looking at the antidiabetic drug

repaglinide

and there again are individuals with the

seal level allele

again homozygotes have higher levels of

repaglinide compared to either

homozygotes or heterozygotes of the

t allele

so thats the second level of evidence

candidate gene studies and really

showing a clinical pharmacology

mechanism behind the statininduced

myopathy

clearly people on simvastatin who have

the seal allele probably have higher

levels of simvastatin as shown in this

particular study over here and those

higher levels put them at risk for

statininduced myopathy

the next level of evidence that we ask

is is really the valine to alanine or

the

ctc

um is that really a functional allele so

these are studies done in the early

000s by richard kim and his group

and what they did here was they studied

the function or the uptake of ester and

sulfate in cells expressing the

reference oatpb

and a whole lot of the variant oatp

oatpbs that they had discovered and

here is whats called the star allele

and the star allele is really the c

tc change or the

valine to alanine change and you can see

theres reduced astron sulfate uptake

compared to the reference alleles of

oatpb

so thats good functional evidence

that this variant causes a reduced

transport rate of estrogen sulfate

secondly they explored the mechanism a

little bit further here as to why it

causes a reduced function and a reduced

transport rate of

in this case ester and sulfate and what

they found using western blot is that

the transporter is not expressed on the

cell surface and im showing you that

here

the transporter is simply not on the

soft surface theres star five where the

reference is really on the cell plasma

membrane so the reason this transporter

is not functioning very well

um

is that its just simply not on the

plasma membrane so those are the third

line of evidence so weve seen

genomewide level of evidence

weve seen several candidate gene

studies showing a clinical pharmacologic

mechanism where individuals who have

this allele homozygous for this allele

have higher levels

of of statins and repaglinide and

finally we show functional evidence

in the literature

that

there is reduced activity of this

particular oatp polymorphism

i now want to go to abc g

so abcg remember includes breast cancer

resistance protein the allele here is a

qk so its changing the amino acid

you call those by the way nonsynonymous

variants or missense variants and im

reminding you that its very striking

for its um

association for its presence if you will

or allele frequency in asian populations

0 is a pretty high allele frequency

um and remember it has met these three

criteria and lets go through those

three criteria for this one

so first reminding you that here it is

in the intestine

and

its also by the way it was also shown

in the liver

on the

canalicular membrane and its also

present in the kidney

on the apical membrane but its a major

intestinal efflux pump and its thought

to limit the bioavailability or the

absorption the extent of absorption of a

number of drugs that are substrates for

it

it transports structurally diverse drugs

including statins antican antigout

drugs and anticancer drugs

and its located on chromosome

all right so lets look first at

genomewide level significance

so in multiple genome association

studies

this particular variant

has been associated with efficacy of

statins

so resuva statin

has a pharmacologic effect if you will

on ldl cholesterol

and individuals who have this variant

have a greater effect of resuvestat

and this shows again were looking at

minus log p value so increase the p

increasing uh peak height or increasing

data points data points at these higher

levels

have lower p values you know p less than

0 to the minus um whatever this this um

this g wass was done in 00

and abc g was highly significant with

um greater efficacy

of um

of receivastatin on ldl cholesterol

and this shows the pvalue 0 to the

minus which is well above genomewide

level significance this is a locus zoom

so youre not looking at all the

different

chromosomes like you were on the oatpb

plot with statininduced myopathy

now in clinical pharmacology mechanistic

studies pharmacokinetic studies if you

look resuvastatin

is

present or its concentrations in

individuals who have the k allele again

who are homozygous recevistatin

concentrations

are higher compared to individuals who

are heterozygous or uh for the reference

allele or are individuals who are um

homozygous for the reference allele so

if you have the variant allele you get

higher drug levels of resuvestatin why

possibly because more resuvastatin is

absorbed that efflux pump cant if

doesnt efflux as well and when you have

this variant when you have the k allele

uh youre not eflexing uh

statin as well and more resuvastatin is

being absorbed i say increase f which

means increased bioavailability of

resuvastan which

accounts for those higher

drug levels of rasuvista

and those of you who are familiar with

fda labeling know that rosuvastatin is

labeled

to give a lower dose of this particular

statin to asians and its probably

although that still is controversial

because asians have a higher allele

frequency of this particular variant so

they

the asians that have this variant will

have higher concentrations of versus

statin so you may want to give them a

lower dose

okay so the question is

is there mechanistic data that suggests

that this particular abc g variant or

bcrp variant

has lower functional activity and so

this is study where they have identified

abc g using an antibody on the plasma

membrane and you can see the reference

transporter is highly expressed on the

plasma membrane shown in green and these

are cells

cell cellular studies however the

variant

transporter has a much lower expression

on the plasma membrane so thats why it

results in reduced function its simply

not on the plasma membrane or less of it

is on the plasma membrane to eflux

the drug and they did further

studies asking the question of why is

less on the plasma membrane and finding

that thats because the

the variant transporter the one with the

lysine at position 0

one

is subject to more increased lysosomal

and proteosomal degradation so just

simply not on the plasma membrane

so those are the lines of evidence for

abc

g and for oatpb so now how do you do

a

study and especially a targeted clinical

mechanistic

pharmacogenomic study

well

we recommend and this is the

international transporter consortiums

paper

but theres a recommendation to collect

dna in all phases of clinical studies

phase phase post marketing collect

dna if you dont have the dna and you

havent appropriately consented the

subjects or the patients

you cant do the pharmacogenomic study

we also recommend some preclinical

studies done first

so lets start with the preclinical

studies what do you do

well the first thing you have to do if

you want to know whether to do a

pharmacogenomic study related to oatpb

or bcrp is ask the question of whether

your drug is actually a substrate of

oatpb or bcrp so here we say do an in

vitro assay in a transfected cell line

expressing oatpb or bcrp and ask

whether its a substrate of the

transporter

if it is a substrate then you move on if

its really not a substrate or if its a

weak substrate and you dont feel that

that transport is playing a major role

or contributing a lot to the disposition

of your new drug

you may not consider a pharmacogenomic

study

we also

next recommend that you consider doing a

drug drug interaction study

so lets say cyclosporine as we know is

an inhibitor and it inhibits many things

but it also inhibits oatpb

and also inhibits oatpb

so its sort of what you might call a

dirty inhibitor

if you do a drug drug interaction study

with cyclosporin you will for sure be

inhibiting oatpb

and also other transporters and if you

get a result that looks like this here

are the plasma levels of your new drug

in the presence and absence of

cyclosporine and theres not much of an

effect

with a drug drug interaction youre

likely not going to get an effect of a

genetic polymorphism if a drug which is

inhibiting in the transporter fully

maybe fully but quite a lot and other

transporters cant cause this drug drug

interaction

youre likely not going to its a small

effect and if especially if you have a

wide therapeutic window dont bother

with the clinical pharmacogenomic study

however if with the drug drug

interaction study you get some clue that

in fact with cyclosporine drug levels go

up that means individuals that carry

genetic polymorphism their levels will

also go up

probably and so now you might want to do

that clinical pharmacogenomic

study

so we think a drug drug interaction

study you may have to do that anyway

because your drug is a substrate of

oatpb and its recommended you do the

ddi

drug drug interaction study so do the

drug drug interaction study first and

get some information and if you get a

large effect and especially if you have

a narrow therapeutic window drug genetic

polymorphisms could play a role in

higher drug levels which may lead to

drug toxicity

you need a strategic design here and we
just and here im not going to prescribe
or describe
clinical pharmacogenomic study design
but when do you do the study do you do
it in phase one do you do it in phase
three do you do post marketing how do
you power the study you have to have
people who are homozygous for that
variant of interest because as you saw
the big effects on drug levels occur
with
in individuals who are homozygous which
ethnic group do you study
certainly if youre looking at the bcrp
variant which is present at high allele
frequencies in east asians you may want
to do the drug drug interaction study in
east asians because youll have a large
population of people who harbor that
genetic variant and then you need an
important data analysis plan and this
definitely requires working with
statisticians geneticists etc for four
of the clinical pharmacogenomics study
so let me review our key points on the

polymorphisms first polymorphisms in

bcrp and oatpb play a role in

variation in drug absorption disposition

and response the international

transporter consortium recommends a

strategic approach to pharmacogenomic

studies where you start in preclinical

cellbased studies and ask simple

questions like is your drug or is it a

drug that you would like to study a

substrate of oatp or bcrp you consider

doing a drug drug interaction study

because you may get information on how

large that effect is going to be and

then you carefully design your

pharmacogenomic study

power it make sure you have homozygotes

etc

so hopefully ive talked about two

genetic variants here in membrane

transporters there are others that are

on the horizon by the way that clinical

pharmacologists will have to be aware of

with time and its very important that

as you look at the data in the

literature and make decisions yourself

on whether a polymorph is is important

you ask some of those fundamental

questions does the polymorphism

associate with drug levels

do

is there

cellular evidence that the polymorphism

is actually causal or could it be in

linkage with a causal polymorphism etc

now let me summarize

the final points in this

in my talk

so first in the area of basic

transporter biology

i want to emphasize two points there are

two major super families slc and abc

that you need to be aware of

that the kinetics of transporters

are

all transporters exhibit saturability

and inhibition

and

the abc transporters can be inhibited

and so can the slc transporters and they

will saturate as well remember that the

slc transporters

are facilitated transporters and do not

rely directly on hydrolysis of atp

they may be secondary active transporter

in which

movement of their substrate is coupled

to the movement of an ion like sodium

and that ion gradient is created by a

transporter which may be directly

dependent on atp hydrolysis like sodium

potassium atpase the abc transporters in

contrast directly rely on hydrolysis of

atp they can actively pump their

substrates against a concentration

gradient and the ones we talk about in

the human genome that are most important

are efflux pumps

transporters may mediate drug drug

interactions and here instead of every

transporter in the world you do have to

be aware of the transporters because

your particular drug may interact with a

transporter that isnt so well known but

the transporters that generally clinical

pharmacologists need to be aware of are

pgp bcrp in the kidney oct octu excuse

me mate mate k 00 and 0 and in the

liver oatpb and v

and maybe at a later date oct but well

see

decision trees are available from the

fda the ema these decision trees really

help you use in vitro studies

to inform the conduct of a clinical drug

drug interaction study and i went

through one but you should go through

other decision trees

in your education as a clinical

pharmacologist

and finally

transporter polymorphisms

play a role in variation in drug safety

drug efficacy

drug

drug levels and i highlighted two

oatpb and bcrp those two particular

polymorphisms however you need to be

aware that there are other polymorphisms

under study and over the course of your

career there will likely be other

polymorphisms

that are

determined and associated with variation

in drug response

finally a strategic design we

recommend a strategic design going

through all phases of drug development

from in vitro or preclinical all the

way

through postmarketing

in the design of a clinical

pharmacogenomics study

i hope this has been a helpful

discussion and lecture on transporter

biology and whats important for

clinical pharmacologists