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Xenobiotika: Interaktionen und Alterationen im humanen Metabolismus.

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1 Zusammenfassung der Publikationspromotion

1.1 Titel und Autor

Xenobiotika: Interaktionen und Alterationen im humanen Metabolismus.

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1.2 Abstract deutsch

Die Zahl der verschriebenen Medikamente steigt stetig und schon heute ist eine Vielzahl von Patienten einer Polypharmazie ausgesetzt. Es treten mit steigender Zahl von applizierten Medikamenten vermehrt unerwünschte Arzneimittelwirkungen (UAW) auf; sogar Todesfälle sind beschrieben. Dabei beeinflussen auch rezeptfreie Präparate und Nahrungsmittel den Metabolismus von Medikamenten. Die Biotransformation ist hauptverantwortlich für die Entgiftung und Elimination von körperfremden Stoffen (Xenobiotika). Die in drei Phasen eingeteilten Reaktionen machen Stoffe hydrophiler und damit besser eliminierbar. An diesen Reaktionen sind hauptsächlich Enzyme der Cytochrom P450 Familie (CYP) und Transferasen beteiligt. In der dritten Phase sind Transportvorgänge durch die hydrophobe Zellmembran zusammengefasst. Enzyme der Biotransformation sind ebenfalls an der Aktivierung von Prodrugs beteiligt. Das System beinhaltet ein großes Potential für Interaktionen, da diese Enzyme überlappende Substratspezifitäten aufweisen. Es kommt zu Inhibierungen und Induktionen der Enzyme durch Xenobiotka und damit zur Beeinflussung anderer gleichzeitig applizierter Wirkstoffe/Substrate. Des Weiteren beeinflussen CYP-Polymorphismen die Enzymaktivität und damit die Biotransformation. Ziel dieser Arbeit war es, eine umfassende Wissensquelle für Medikamentenmetabolsierungen und deren Einflussfaktoren zu schaffen. Zur Kollektion der Informationen wurde ein Textming von 21 Millionen PubMed Abstracts durchgeführt. Damit konnten CYP Polymorphismen, Phase I, II und III Interaktionen, Prodrugs und deren Mechanismus der Aktivierung extrahiert werden. Zusätzlich wurden durch eine Expressionsanalyse von CYPs Hinweise für eine heterogene Verteilung dieser Enzyme im menschlichen Körper gefunden. Eine im Internet veröffentlichte Datenbank bietet der wissenschaftlichen Gemeinschaft umfassendes Wissen zur Metabolisierung von 2.804 Wirkstoffen und beinhaltet dabei über 100.000 mögliche Interaktionen. Die Visualisierung der Interaktionen wird durch eine Auswahl an alternativen Medikamenten ergänzt, mit denen das Interaktionspotential gesenkt werden könnte. Eine Analyse mithilfe dieser Datensätze deckte mögliche Interaktionen bei Chemotherapieprotokollen in der Kinderonkologie auf. Daten dieser Arbeit sollen eine Ressource und Fundament für die personalisierte Medizin sein und eine Möglichkeit bieten, Polypharmazie besser zu bewältigen. Durch die Betrachtung von Einflussfaktoren könnte die Zahl der UAWs und der Therapieversager gesenkt werden.

1.3 Abstract englisch

The number of prescribed drugs is rising constantly. Nowadays, many patients are at risk as a result of polypharmacy. The occurrence of undesirable side effects becomes larger with increasing drug-intake; even fatal adverse effects have been described. Thereby, over-the-counter drugs and food also have an effect on drug metabolism. Biotransformation is mainly responsible for the detoxification and elimination of exogenous substances (Xenobiotics). Subdivided into three reactions, biotransformation ensures that chemicals become hydrophilic and, as a consequence, are easier to eliminate. The primary enzymes involved are the Cytochrome P450 enzymes and transferases. The third phase reflects the transport through the hydrophobic cell membrane. In addition the enzymes of the biotransformation take also part in activation of prodrugs. This system contains great potential for interactions, because of the overlapping substrate specificity. Two different types of interaction can occur: inductions and inhibitions. This can have an effect on other drugs, when administered at the same time. Furthermore CYP-polymorphisms can alter the enzyme activity and thus the biotransformation. The aim of this research was to build up a comprehensive source of drug metabolizing knowledge with special consideration of factors which impair metabolization. To collect this information, a text-mining approach of 21 million PubMed abstracts was used. Thus, articles containing the keywords CYP polymorphism, phase I, II and III interactions and prodrugs (with their mechanism of activation) could be extracted. In addition, an expression analysis revealed indications for the heterogeneous distribution of CYPs in humans. The results of these investigations are stored in a database and are publicly available through the internet. This database offers the scientific community comprehensive information about the metabolization of 2,804 drugs and thereby contains over 100,000 possible interactions. The visualization of interactions of a drug compound is supplemented by alternative drugs, which have lesser interactions. An analysis with the help of these findings revealed possible interactions at child oncology chemotherapy regimens. Data of this work should represent a resource and fundamental basis for personalized medicine, whilst also providing an opportunity to have better control in polypharmacy. Through the consideration of influences, the number of UAWs and non-responders could be reduced.

1.4 Einleitung

Biotransformation

Xenobiotika sind körperfremde Stoffe, welche definitionsgemäß nicht vom menschlichen Organismus synthetisiert werden können. Eine bedeutende Untergruppe stellen Medikamente dar, aber auch in Nahrungsmitteln finden sich vielfach Xenobiotika. Ein Teil dieser Stoffe besitzt toxische Eigenschaften und kann nicht ohne weiteres eliminiert werden. Daher ist der menschliche Körper gezwungen, sich mit diesen Stoffen auseinanderzusetzten. Die Detoxifikation und Eliminierung kann in drei Phasen eingeteilt werden und wird als Biotransformation bezeichnet. In der ersten Phase werden funktionelle Gruppen eingefügt oder reaktive Gruppen geschaffen. Diese Reaktionen werden vornehmlich durch die Cytochrom P450 Enzymfamilie (CYP) realisiert [1]. Das Human Genome Projekt identifizierte 57 humane CYPs und teilte sie in 18 Familien und 43 Subfamilien [2]. Die zweite Phase nutzt funktionelle Gruppen und konjugiert diese mit kleinen polaren Molekülen. Dadurch wird die Polarität des Stoffes erhöht. Dazu stehen sechs Enzymfamilien zu Verfügung, z.B. Methyltransferasen, Sulfotransferasen und Acetyltransferasen [3]. Zweck dieser Reaktionen ist es einerseits, Stoffe hydrophiler zu machen und damit besser ausscheiden zu können und andererseits, toxische Stoffe zu entgiften. Dabei müssen die Stoffe nicht zwangsläufig beide Phasen durchlaufen. Da die Enzyme intrazellulär lokalisiert sind, muss ein Durchtritt durch Zellmembranen gewährleistet sein. Dies wird in der dritten Phase abgebildet und durch Transporter realisiert. Es gibt zwei Gruppen von Transportern: 49 *ATP-binding cassette* (ABC) Transporter [4] und 362 *solute carriers* (SLC) Transporter [5]. Erstgenannte verbrauchen Energie für den Transport, wohingegen die SLC einen passiven Durchtritt durch die Zellmembran ermöglichen. In Abbildung 1 wird die Biotransformation am Bespiel von Propranolol veranschaulicht.

Prodrugs

Ein weiterer Bereich, der bei der Metabolisierung von Xenobiotika eine Rolle spielt, ist die Aktivierung von Prodrugs. Prodrugs sind Medikamente, welche im Körper erst in ihre aktive Form überführt werden müssen. Dabei gibt es Medikamente, die durch Änderung des pH-Wertes (z.B. Omeprazol) [6] oder durch Enzyme zu ihrem aktiven Metaboliten werden. Als Beispiele seien hier die Aktivierung von Ramipril zu Ramiprilat via einer Esterase [7] oder Cyclophosphamide via CYP2B6 und CYP3A4 zu 4-Hydroxy-cyclophosphamid zu nennen [8].

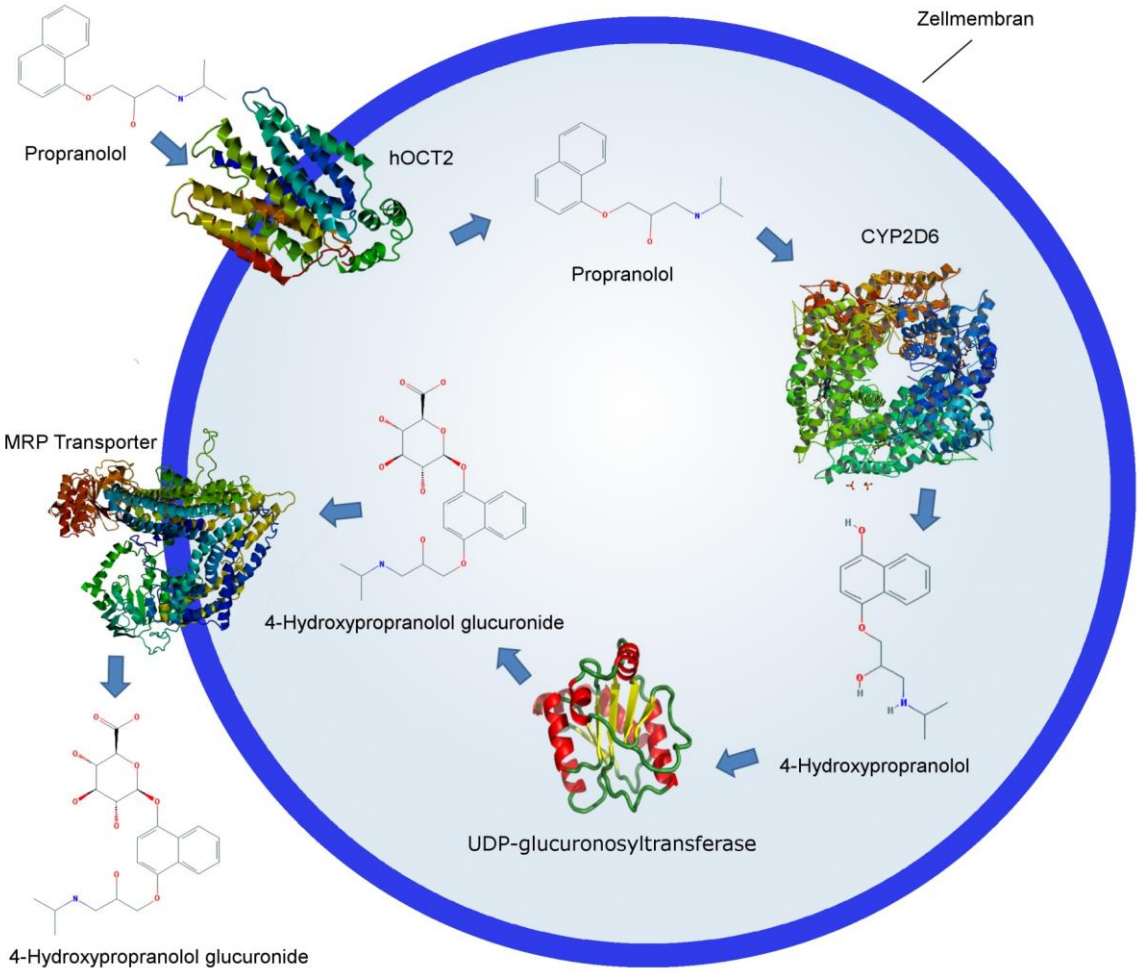

Abbildung 1: Die Biotransformation von Propranolol. Der *Organic cation transporter 2* (hOCT2, Protein Data Bank (PDB)-Code: 4gc0A) transportiert den Stoff in die Zelle [9]. Anschließend findet eine Hydroxlierung durch das CYP2D6 (PDB-Code: 2F9Q) [10] und eine Glucuronsäurekonjugation mit Hilfe der Uridin 5'-diphospho-glucuronosyltransferase (UDP-glucuronyltransferase, PDB-Code: 2o6lA) [11] statt. Der Efflux wird von einem Glucuronid-Transporter der *Multidrug resistance protein* (MRP) Familie realisiert. In diesem Beispiel wurde dafür exemplarisch der MRP1 (PDB-Code: 4f4cA) Transporter gewählt [12]. Die chemischen Strukturen wurden PubChem (pubchem.ncbi.nlm.nih.gov/) entnommen.

Alterationen in der Biotransformation

Es kann zu Interaktionen im Metabolismus von Xenobiotika kommen und dadurch deren Abbau oder Aktivierung beeinflusst werden. Dabei treten Inhibierungen von Enzymen durch Xenobiotika auf, welche die Metabolisierungsrate anderer Substrate vermindern. Auf der anderen Seite kann es zur Induktion von Enzymen und damit zu einer höheren Umsatzrate des Substrates kommen. Eine weitere Form der Interaktion ist die Kongruenz zweier Substrate um das gleiche Enzym. In der Pharmakotherapie spielen

diese Beeinflussungen eine besondere Rolle. Es wurde zum Beispiel gezeigt, dass sich durch die gleichzeitige Applikation von Atorvastatin (ein HMG-CoA-Reduktasehemmer) zusammen mit Clarithromycin (einem Makrolidantibiotikum), die 24h *area under the curve* (Bioverfügbarkeit) von Atorvastatin um 82,0% vergrößerte und der Spitzenwert im Plasma um 56,0% angehoben wurde [13]. Kausal liegt dieser Beobachtung eine Inhibierung von CYP3A4 durch Clarithromycin und damit einem geringerem Abbau von Atorvastatin zugrunde. Diese Augmentation des Atorvastatins steigert die Wahrscheinlichkeit einer unerwünschten Arzneimittelwirkung (UAW), in diesem Beispiel wäre dies eine Rhabdomyolyse [13]. Die Inzidenz von UAWs bei hospitalisierten Patienten beträgt 6,7% [14] und steigt mit der Anzahl der applizierten Medikamente [15].

Polymorphismus

Allerdings nicht nur Medikamente untereinander beeinflussen die Biotransformation. Der Polymorphismus von Enzymen ist eine Ursache für die interindividuelle Variabilität in der Metabolisierung von Medikamenten. Dabei können Einzelnukleotid-Polymorphismen (SNP, Englisch: single nucleotide polymorphism) zu einem Verlust, Abschwächungen oder Verstärkung der Enzymaktivität führen. So führt der CYP2C19*2 Polymorphismus zu einer geringen Aktivität dieses CYPs und tritt mit einer Häufigkeit von 16,0% unter Kaukasiern auf. Clopidogrel (ein Thrombozytenaggregationshemmer via Inhibierung des ADP- P2Y12-Rezeptors) ist ein Prodrug und benötigt die Aktivierung durch CYP2C19. Eine Metaanalyse zeigte, dass CYP2C19*2 Träger, die mit Clopidogrel behandelt wurden, ein um 30,0% höheres Risiko für kardiovaskuläre Ischämien aufwiesen [16]. Daher liegt die Vermutung nahe, dass in diesen Fällen die Wirkung des Clopidogrel nicht ausreichte und es nicht adäquat aktiviert wurde.

Bedeutung in der Medizin

Das System der Xenobiotika/Medikamenten Metabolisierung ist sehr umfangreich. Die beteiligten Enzyme zeigen überlappende Substratspezifitäten, die Polymorphismen sind in jeder Ethnie unterschiedlich verteilt und die Xenobiotika können die Enzymaktivität in unterschiedlicher Weise modifizieren. Hinzu kommen, die Zunahme der applizierten Medikamente pro Patient [17] und der demographische Wandel [18], welcher die Zahl der multimorbiden Patienten und damit verbunden der Polypharmazie weiter vergrößern wird. Die Prävalenz der Menschen über 50 Jahre, die mindestens ein Medikament pro Tage einnehmen, betrug im Jahr 2009 in Australien 87,1%, 43,3% nahmen sogar mehr

als fünf Medikamente ein [19]. Um nun mögliche UAWs bei diesen Polypharmaziepatienten vorzubeugen respektive zu verhindern, ist es wichtig, eventuell vorkommende Interaktionen vorhersagen zu können. Dafür wäre ein umfassendes Wissen über die Metabolisierung und Interaktionen der einzelnen Stoffe notwendig. Des Weiteren sollte die individuelle Enzymaktivität berücksichtigt werden und Polymorphismen in der Gesamtbetrachtung nicht fehlen. Leider sind Informationen zur Biotransformation und Polymorphismen nur weit verstreut in der wissenschaftlichen Literatur zu finden.

Tabelle 1: Beispiele für Medikamenteninteraktionen, die in klinischen Studien und Fällen beobachtet wurden; AUC – Fläche der Konzentrations-Zeit Kurve.

Xenobiotika	Beeinflussung	Beobachtete Auswirkung
Erythromycin	CYP3A4 Inhibitor	Die AUC von Simvastatin wurde um das 6,2-fache größer und die Spitzenserumkonzentration um das 3,2-fache angehoben [20]
Omeprazol	CYP2C19 Inhibitor	Verlängerung der Halbwertzeit von Diazepam in extensiv Metabolisierer [21]
Amlodipin	CYP3A4 Inhibitor	Erhöhung des Risikos von thrombotischen Ereignissen nach einer perkutanen Koronarintervention unter der Prophylaxe von Clopidogrel [22]
Paroxetin	CYP2D6 Inhibitor	Verminderung der Wirkung von Tamoxifen [23]
Fluoxetin	CYP2D6 Inhibitor	Assoziation mit tödlich verlaufenden Codeinintoxikationen und post mortem erhöhten Codein- und Morphin-Konzentrationen [24]
Grapefruchtsaft	CYP3A4 Inhibitor	Vergrößerung der toxischen Wirkungen von Amiodaron; steigendes Risiko für ventrikuläre Tachykardien, Torsade de pointes, QT-Zeitverlängerungen [25]
Rifampicin	CYP3A4 Induzierer	Die AUC wurde bei intravenöser und oraler Oxycodone Applikation um 53,0% und 86,0% reduziert [26]
Omeprazol	CYP2C19 Inhibitor	Reduzierung der Wirkung von Clopidogrel [27]
Carbamazepin	CYP3A4 Induzierer	Reduzierung des Plasmalevels von S-Citalopram und R-Citalopram um 27,0 % und 31,0 % [28]
Atorvastatin	P-Glycoprotein Inhibitor	Die AUC wurde um 15,0 % vergrößert und die maximale Plasmakonzentration um 20,0 % [29]
Verapamil	P-Glycoprotein Inhibitor	1,8-fache Anhebung der Spitzenplasmakonzentration und Vergrößerung der AUC um das 2,0-fache [30]

1.5 Zielstellung

Ziel dieser Promotionsarbeit war es, Informationen zur Metabolisierung von Medikamenten und deren Interaktionen zusammenzutragen. Dabei sollten diese Daten dann so aufbereitet werden, dass sie jedem in Sekunden zur Verfügung stehen und die Möglichkeit bieten, Wechselwirkungen bei der Metabolisierung zu erkennen und zu umgehen. Dafür wurden folgende Einflussfaktoren bei der Verstoffwechselung untersucht.

1. Medikamenten-Enzym-Interaktionen bei der Biotransformation (Phase I, II & III)
2. Aktivierung von Prodrugs
3. Einfluss von Nahrungsmitteln auf das Cytochrom P450 System
4. Genetische Veränderungen der Enzymaktivität in Form von Polymorphismen
5. Diversität der Cytochrom P450 im menschlichen Körper

Um die Medikamenten-Interaktionsdaten frei zugänglich, übersichtlich und für den Benutzer individuell angepasst darstellen zu können, wurde eine Internetseite konzipiert. Diese visualisiert den Inhalt der Datenbank und erlaubt dem Benutzer mit Daten zu interagieren. Mögliche Folgen einer Kombination von Medikamenten oder alimentär bedingte Interaktionen sollen in Sekunden veranschaulicht werden. Durch eine Auswahl der angebotenen alternativen Medikamente könnten diese Beeinflussungen dann vermieden werden. Die Umgebung ist für Ärzte und Wissenschaftler gedacht, um bei ihrer Forschung mögliche Interaktionen bei der Pharmakotherapie aufzudecken oder eine Erklärung für UAWs zu finden. Dadurch ließe sich in Zukunft die Zahl der UAWs und der Patienten, die nicht auf ein Medikament ansprechen, reduzieren. Unterschiedliche Effekte von Medikamenten in bestimmten Geweben, könnten auch durch die Diversität von CYPs im menschlichen Körper beeinflusst werden. Daher wurden Daten einer Expressionsanalyse untersucht, um Hinweise für eine heterogene Verteilung der CYPs zu finden. Des Weiteren sollte eine Suche nach den relevantesten CYP Polymorphismen, welche häufig für die intraindividuelle Medikamentenantwort verantwortlich sind, ein weiteres Werkzeug bei der Vorhersage von UAWs und verminderter Medikamentenwirkung sein. Für eine erste praktische Anwendung der gesammelten Daten wurden Chemotherapieschemata auf ihre CYP-Interaktionen analysiert. Bei steigender Zahl von Polyphamaziepatienten ist es Ziel dieser Arbeit, UAWs und ein Nicht-Ansprechen auf Wirkstoffe zu verhindern und bei der Vielzahl von Interaktionsmöglichkeiten einen Überblick zu geben.

1.6 Methodik

1.6.1 Textmining

Für alle drei Publikationen wurde diese Art der Analyse durchgeführt. Textmining ist ein Analyseverfahren von Texten, um automatisiert definierte Inhalte zu erfassen. Die Grundlage dafür sind Listen mit sogenannten Schlagwörtern und Beziehungswörtern. Es wird nach diesen Schlagwörtern in Texten gesucht und deren Beziehung zueinander evaluiert. In dem Fall der Enzym-Medikamenten-Interaktionen waren Schlagwörter Enzyme und Medikamente plus deren Synonyme. Die Beziehungen wurden durch Begriffe determiniert, welche eine Interaktion zwischen den Schlagwörtern darstellen, z.B. Substrat oder Inhibitor. Zur Umsetzung dieser Herangehensweise wurden 21 Millionen Abstracts von PubMed im XML Format von deren FTP-Server heruntergeladen. Mit Hilfe der Apache Lucene Programmbibliothek (http://lucene.apache.org) war es möglich, diese Daten zu indizieren. Anschließend konnte unter Zuhilfenahme der LingPipe Java-Bibliothek (http://alias-i.com/lingpipe) ein Java Programm erstellt werden, welches die Suche nach relevanten Beziehungen in dem Datensatz ermöglichte. Diese Ergebnisse wurden dann in einem Browsertool manuell überprüft und anschließend die Enzyme-Substrat Beziehung in die Datenbank überführt. Um die falsch-positiv-Rate zu minimieren, wurde ein Score (0 bis 100) berechnet, der den Abstand der Schlagwörter zum Beziehungswort bewertete. So wurden nur Abstracts mit einem Score größer 50 berücksichtigt. Dies verringerte zum Beispiel die Anzahl der relevanten Abstracts beim Textmining für die Transformer Datenbank von 22.500 auf 12.427.

Tabelle 2: Übersicht der beim Textmining gesuchten Biotransformationsenzyme.

Phase I	Phase II	Phase III
57 Cytochrom P450 Enzyme	Acetyltransferase	49 *ATP-binding cassette* Transporter
	Glutathion S-transferase	362 *solute carrier* Transporter
	Methyltransferase	
	N-acetyltransferase	
	Sulfotransferase	
	UDP-glucuronosyltransferase	

Biotransformations Interaktionen

Schlagwörter setzten sich bei dieser Suche aus 2.802 Xenobiotika (Medikamente und Nahrungsmittel) und den in Tabelle 2 dargestellten Enzymen zusammen. Die Schlagwortlisten wurden zusätzlich um die jeweiligen Synonyme erweitert. Für die Beziehungen wurden folgende Wörter definiert: *inhibitior* / *inhibition*, *inducer* / *induction*, *substrate*, *decrease*, *increase*, *metabolize* und *activate*.

Prodrugs

Bei diesem Textmining wurden die Medikamente unserer Datenbank mit Beziehungswörtern gesucht, welche eine mögliche Umwandlung zu einem aktiven Wirkstoff bedeuten könnten. So wurden Begriffe wie *active metabolite*, *activation*, *mechanism of activation*, *active drug*, *converted* and *bioactivation* gewählt. Leider wurden nicht immer alle Informationen in den Abstracts abgebildet. Dies machte zusätzlich eine intensive manuelle Volltextsuche erforderlich.

Chemotherapie Interaktionen

Es wurde nach 57 CYPs und Wirkstoffen der Therapieschemata gesucht, dabei entsprachen die Beziehungswörter denen des Textmining der Biotransformation. Die Ergebnisse dieser Analyse wurden in die Biotransformationsdatenbank übernommen.

Polymorphismen der Cytochrom P450

Die Nomenklatur der CYP Allele ist durch Zahlen definiert, welche dem CYP Namen hinten angestellt und durch ein Sternchen (*) getrennt (z.B. CYP2A6*2) werden. Als Schlüsselwörter wurden daher die CYPs gefolgt von einem Sternchen gewählt, ohne nach bestimmten Allelen zu suchen. Weiterhin sollte eine Verbindung zu den Begriffen *population*, *effect* und *frequency* bestehen. Leider befanden sich diese Informationen nicht alle im Abstract und es musste im Anschluss eine manuelle Volltextsuche erfolgen. Der Fokus wurde bei dieser Analyse auf Kaukasier gelegt. Wenn dennoch Informationen zu anderen Ethnien angegeben waren, wurden diese ebenfalls mit erfasst.

1.6.2 Expressionsanalyse

Um die Diversität der CYPs in menschlichen Geweben zu untersuchen, wurde ein Expressionsdatensatz benötigt. Dieser musste verschiedene Gewebearten beinhalten und durfte nicht durch eine Pathologie beeinflusst worden sein. Die Serie GSE3526 aus der GEO Datenbank (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) erfüllte diese Kriterien und wurde aus Gewebeproben von 10 (5 weiblich, 5 männlich) post mortem Spendern generiert. Dabei wurden aus 65 Regionen 41 verschiedene Gewebearten erfasst und insgesamt 353 Proben gewonnen. Die Expressionslevel wurden mit dem Human Genome U133 Plus 2.0 Array gemessen. Dieser beinhaltet unter anderem 84 Expressionslevel, welche CYPs zugeordnet werden können. Führt man diese zu den jeweiligen Isoformen zusammen, ergibt dies 40 verschiedene CYPs. Nachdem die Daten kondensiert wurden, konnte eine Datei für das Programm Genesis [31] erstellt werden. Das Programm wurde dazu verwendet, eine *heat map* zu erstellen. Eine *heat map* ist ein Diagramm bei der die Höhe eines Wertes farbcodiert wird. So werden markante Werte in einer großen Anzahl von Daten augenscheinlicher. Dafür musste eine Z-Score-Berechnung erfolgen, bei der die jeweiligen CYP-Expressionslevel vom Mittelwert aller Level in einem Gewebe subtrahiert werden. Anschließend wurde dieser Wert durch die Standardabweichung geteilt. Durch diese Berechnung lassen sich die Expressionslevel leichter vergleichen [32]. Wie in Abbildung 2 zu sehen ist, konnten darauffolgend die Unterschiede der Expressionsniveaus in der *heat map* visualisiert werden. Die Intensität der Farben spiegelt dabei die Größe des Unterschiedes wieder. Die Ergebnisse wurden anschließend in die CYP-Körperkarte übertragen. Es wurden in dieser Karte nur Werte visualisiert, die um mindestens zwei Standardabweichungen vom Mittelwert divergieren. Somit werden nur große Unterschiede berücksichtigt. Diese Analyse soll nur Hinweise auf eine unterschiedliche Verteilung der CYP liefern und erlaubt keine absoluten Aussagen über die unterschiedliche Expression von CYPs beim Menschen. Jedoch die Darstellung der am weitesten vom Mittelwert entfernten Ergebnisse (repräsentiert durch 4,6 % aller Daten) kann Hinweise geben, welche die Diversität der CYPs im menschlichen Körper untermauern. Wir danken der Arbeitsgruppe des Department of Molecular Medicine in San Diego (USA) [33] für die Erhebung und Bereitstellung dieser Daten.

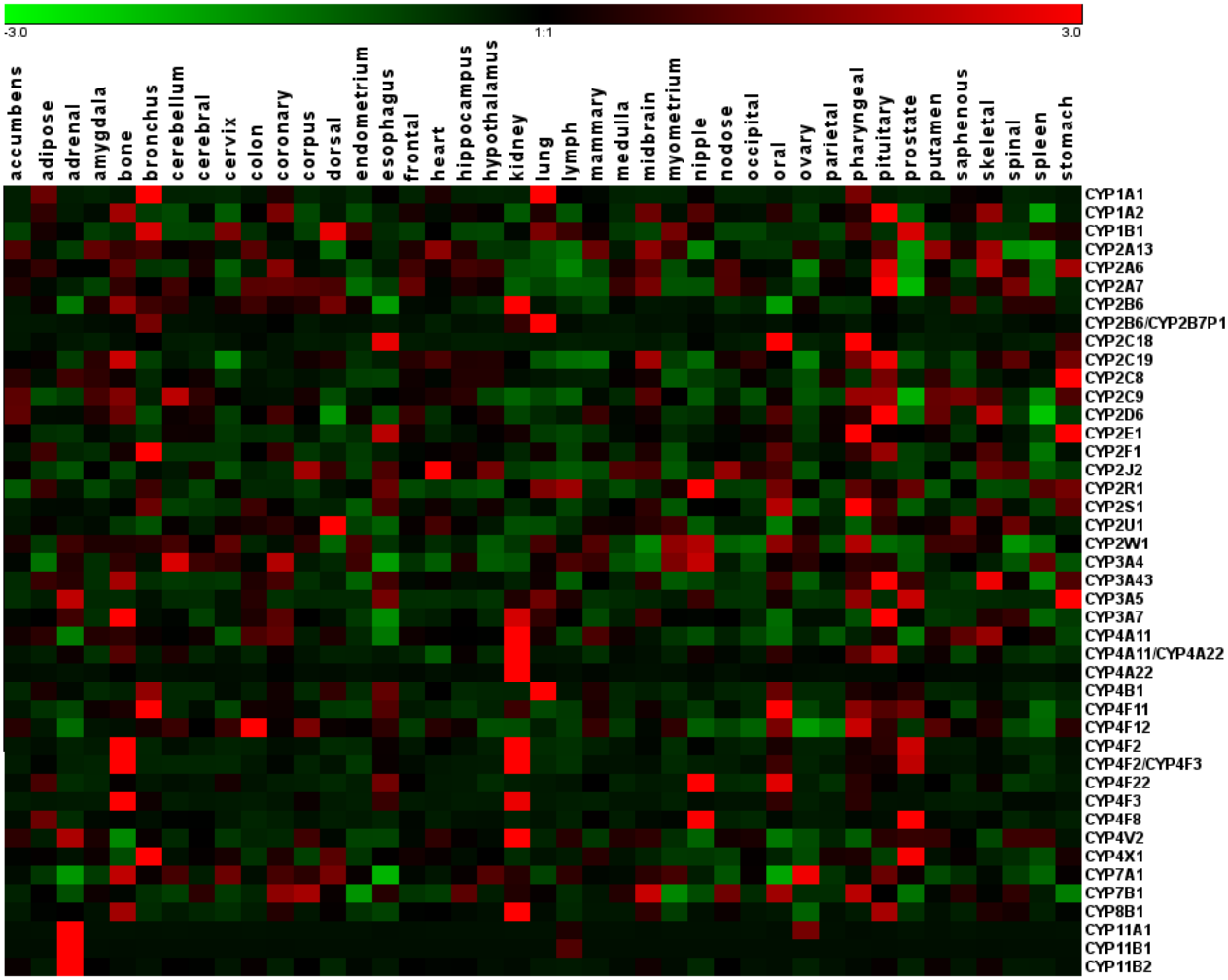

Abbildung 2: *Heat map* der Expressionsanalyse von 41 humanen Gewebearten und 40 CYP Isoformen.

1.6.3 CYP Polymorphismen aus 1.000 Genomen

Es wurden Daten des 1000 Genomes Project (http://www.1000genomes.org) verwendet und auf SNPs von 45 CYPs untersucht. Dafür wurde das Browsertool der 1000 Genomes Project Internetseite eingesetzt. Die Analyse fokussierte sich auf non-synonymous-kodierende SNPs mit einer Frequenz über einem Prozent in der Bevölkerung (keine Sortierung nach Ethnien). Die potenziellen Auswirkungen auf die Aktivität der CYPs wurden dabei mitberücksichtigt. Diese Effekte konnten mit Hilfe von PolyPhen [34] berechnet werden und prognostizieren mögliche funktionelle Enzymveränderungen nach einem Aminosäureaustausch. In den Datensätzen des 1000 Genom Projektes waren diese Berechnungen schon enthalten.

1.7 Ergebnisse

Biotransformation und Datenbank

Es konnte für 769 Medikamente eine Interaktion mit Phase II Enzymen zugeordnet werden. Weiterhin wurden zu 150 Nahrungsmittel und 124 Transportern Interaktionen mit Wirkstoffen gefunden. Die Datenbank beinhaltet zudem 125 Prodrugs, mit dem jeweiligen Mechanismus der Aktivierung, dem beteiligten Enzym und dem aktiven Metaboliten. Zusätzlich wurden die dazugehörigen 2D Strukturen in der Datenbank abgelegt. Damit lässt sich der Mechanismus der Aktivierung auf der Internetseite visualisieren. Zu jedem Biotransformationsenzym wurde eine 3D Struktur hinterlegt und sowohl Enzyme als auch Xenobiotika mit gängigen Identifikationsnummern ausgestattet. Die CYP-Interaktionen aus dem Chemotherapie Textmining (723 gefundene Artikel) sowie 3.000 Medikamenten-Interaktionen aus der vorher bestandenen SuperCYP Datenbank [35] konnten in die neue Datenbank integriert werden. Damit beinhaltet die Datenbank über 100.000 mögliche Interaktionen von 2.802 Medikamenten. Eine Übersicht der Datenbankeinträge gibt Tabelle 2.

Tabelle 2: Kategorien in der Datenbank mit Anzahl der eingetragenen Interaktionen.

Datenbankgruppe	Anzahl der Interaktionen
Phase I	4.008
Phase II	431
Transporter	1.158
Nahrungsmittel	350
Referenzen	105.000

Funktionalität der Internetseite Transformer

Die Internetseite Transformer (http://bioinformatics.charite.de/transformer/) bietet die Möglichkeit, Informationen der Datenbank in unterschiedlichster Weise zu visualisieren. So ist es möglich, alle interagierenden Medikamente zu einem bestimmten Enzym und andersherum alle Enzyme zu einem ausgewählten Medikament anzeigen zu lassen. Der Benutzer hat ebenfalls die Möglichkeit, mit Daten zu interagieren. Um zum Beispiel mögliche Wechselwirkungen bei der Applikation von mehreren Medikamenten zu visualisieren, gibt es das „Cocktail Tool“. Dort werden mehrere Medikamente ausgewählt und anschließend ein Überblick über mögliche Interaktionen gegeben. Wenn mehr als zwei Medikamente mit dem gleichen Enzym interagieren, wird dies gelb,

ab drei orange und bei mehr als vier rot unterlegt. Zusätzlich werden dem Benutzer die Eliminationshalbwertzeit und der Q_0-Wert angezeigt. Damit kann der Einfluss der Metabolisierung auf die Elimination abgeschätzt werden. Folgend zeichnet sich dieses Werkzeug dadurch aus, dass es die Auswahl an Alternativen an Medikamenten ermöglicht. Es werden alternative Medikamente aus der gleichen ATC-Gruppe (ATC - Anatomisch-therapeutisch-chemische Klassifikationssystem) angeboten. Dies eröffnet die Möglichkeit, ein Medikament auszuwählen, welches in der Komposition von Wirkstoffen weniger Interaktionen verursacht. Eine weitere Darstellungsart wird in Form eines Netzwerkes angeboten, in dem ausgewählte Enzyme und deren wechselwirkende Medikamente in einer 2D Netzwerkdarstellung visualisiert werden.

Polymorphismen von Cytochrom P450

Die Suche nach Polymorphismen bei Kaukasiern führte zu dem Ergebnis, dass 34 Allele einen Einfluss auf die Aktivität von CYPs in dieser Ethnie haben. Dabei wurden der Definition folgend, nur Allele berücksichtigt, die eine Häufigkeit über einem Prozent aufwiesen. Es zeigte sich, dass das CYP2D6 und CYP2B6 die meisten Allele besitzen. Die Allele mit der größten Frequenz waren dagegen CYP3A5*3C (geringere Enzymaktivität) mit 81,3% und CYP1A2*1F (erhöhte Enzymaktivität) mit 33,3%. Um den Einfluss dieser Polymorphismen auf den Medikamentenmetabolismus in der klinischen Praxis abschätzen können, wurde in der Datenbank die Anzahl der jeweiligen Substrate für CYPs extrahiert. Daraus konnte abgeleitet werden, dass CYP1A2, 2D6, 2C9, 2C19 zusammen für 40,0% der Verstoffwechselung verantwortlich sind und CYP3A4 für 20,0%. Da die vier erstgenannten CYPs auch entweder hochfrequente oder eine große Anzahl von Allelen besitzen, schlussfolgern wir, dass Allele dieser vier CYPs die größte Rolle bei individuellen Veränderungen im Abbau oder Aktivierung von Medikamenten spielen. Des Weiteren konnten Unterschiede zwischen den einzelnen Ethnien aufgedeckt werden. Bei der Suche in 1000 Genomen, konnten 199 non-synonymus-kodierende SNPs detektiert werden, die mit einer Häufigkeit von über einem Prozent auftreten. Wenn die mögliche Auswirkung des Aminosäureaustausches berücksichtigt wird und nur SNPs mit einem wahrscheinlichen Einfluss auf die Enzymaktivität gezählt werden, sind dies 72 SNPs. Bei einem Vergleich mit der Human Cytochrome P450 Allele Nomenclature Datenbank (http://www.cypalleles.ki.se, [36]) wurden viele dieser SNPs nicht in deren Datensätzen gefunden. Kürzlich erfuhr diese Datenbank allerdings ein Update [37].

Expressionsanalyse

Das Ergebnis der Analyse lässt auf eine heterogene Verteilung der CYPs schließen. Es wurden bei 21 CYPs divergierende Expressionslevel gefunden, die sich um mindestens das 2-fache der Standardabweichungen (SD) von anderen unterscheiden. Diese Arbeit konnte die Ergebnisse von Nishimura et al. [38] bestätigen und erweiterte das Bild der Verteilung der CYPs im Körper. So wurden 10 CYP Isoformen und 30 Gewebearten mehr untersucht. Beispielhaft werden folgend einige Gewebe beleuchtet: in der Niere fanden sich 6-fach erhöhte Standardabweichungen für CYP4A22, 5-fach für CYP 8B1, 4-fach für CYP4V2, CYP4F2, CYP4A11 und CYP2B6. Die Lunge zeigte hingegen eine 5-fach erhöhte Standardabweichung für CYP2C8. Interessanterweise zeigte CYP2C18 nur im Cavum oris, Pharynx und Ösophagus eine erhöhte Expression. Harmonisch zur Biochemie der Nebenniere fanden sich dort erhöhte Expressionswerte (6-fache der SD) für CYPs der Steroide und Aldosteron Synthese (CYP11A1, CYP11B1, CYP11B2).

1.8 Diskussion

Die Zahl der wissenschaftlichen Daten und neuen Erkenntnisse wächst stetig. Die Frage ist dabei, wie mit diesen Unmengen von Informationen adäquat umgegangen werden kann. Wenn zum Beispiel die Medikation eines Patienten auf Interaktionen überprüft werden soll und die Angaben auf Beipackzetteln nicht ausreichen, weil vermutet wird, es könnte sich um eine noch nicht beschriebene oder nicht so häufige Interaktion handeln, stehen Informationen verstreut in über 100.000 Abstracts bei PubMed zur Verfügung. Trotz der Suchfunktion wäre dies eine zeitintensive und mühsame Arbeit mit offenem Ergebnis. Aus diesem Grund haben sich Datenbanken etabliert, die tabellarische Informationen enthalten und dem Benutzer die gesuchten Informationen in Sekunden bereitstellen. Das Verfahren ein Textmining durchzuführen und anschließend deren Ergebnisse aufbereitet in einer Datenbank zu speichern, zielt darauf ab, Informationen zu einem bestimmten Themenbereich aus der Vielzahl von Publikationen zu extrahieren und konzentriert an einem Ort abzulegen. So wird einerseits die Möglichkeit geschaffen, in kürzester Zeit einen Überblick zu bekommen und andererseits mit der Verlinkung auf die jeweilige Quelle, die Chance auf detaillierte Informationen nicht vergeben. Dabei schließt die Datenbank Transformer eine bestehende Lücke, wenn es um Informationen zur Biotransformation von Xenobiotika geht. Es gibt schon einige Datenbanken, die sich mit Medikamenten und deren

Beziehung zu Enzymen befassen. Tabelle 3 zeigt einen Vergleich der Transformer Datenbank mit anderen Datenbanken aus diesem Forschungsbereich. Diese Tabelle verdeutlicht, dass andere Datenbanken die drei Phasen der Biotransformation nicht so umfassend abdecken. Ein Grund hierfür ist die Tatsache, dass sie ursprünglich für andere Zwecke konzipiert wurden und niemand die Intention hatte, die Biotransformation im Ganzen abzubilden.

Tabelle 3: Vergleich von Transformer mit ähnlichen Datenbanken. (Stand: Juli 2014)
k.A. – die Statistik der Datenbank macht dazu keine Angaben. +/- Einträge ja/nein.

Datenbanken	Transformer	SuperCYP	DrugBank	KEGG	HMTD
Medikamente	2.802	2.000	1.037	735	67
Referenzen	+	+	-	-	-
Prodrugs	+	-	k.A.	-	-
Nahrungsmittel	+	-	k.A.	-	-
Cytochrom P450	+	+	k.A.	k.A.	-
Phase II	+	-	k.A.	k.A.	-
Transporter	+	-	+	k.A.	+
Interaktionen	über 100.000	k.A.	14.150	14.441	k.A.

Es wurden in der Vergangenheit immer mehr Medikamenteninteraktionen aufgedeckt, bei denen Biotransformationsenzyme eine Rolle spielen. Sei es durch die gegenseitige Beeinflussung von Medikamenten durch das Interagieren mit einem Enzym oder durch hereditäre Veränderungen in der Enzymkinetik. Möchte man herausfinden, warum ein Patient anders auf ein Medikament reagiert, sollte das Interesse auch der Metabolisierung von Medikamenten gelten. Ist eine falsche Applikation ausgeschlossen, könnte dieser Patient zum Beispiel einen Polymorphismus tragen, welcher ein Medikament schneller eliminiert oder ein Prodrug vermehrt aktiviert. Anderseits könnte die Begleitmedikation ein Biotransformationsenzym induzieren oder inhibieren, welches für die Metabolisierung des Medikamentes zuständig ist. Die relevanten Polymorphismen lassen sich mit Hilfe eines DNA-Chips untersuchen und Interaktionen werden durch die Transformer Datenbank aufgedeckt. Limitiert werden diese Aussagen durch die begrenzte Substratspezifität der beteiligten Enzyme. So ist es bei einem beeinträchtigten Metabolisierungsweg über ein CYP möglich, dass ein anderes CYP diese Aufgabe vermehrt übernimmt und ein Effekt nicht beobachtet wird. Daher führt eine Interaktion nicht zwangsläufig zu einer veränderten Gesamtmetabolisierung eines Medikamentes. Dies wird von der Tatsache untermauert, dass in der Datenbank ein

Medikament des Öfteren Substrat für mehrere CYPs ist. Auf der anderen Seite konnten, wie in Tabelle 1 dargestellt, im klinischen Alltag und im Labor schon zahlreiche Interaktionen beobachtet werden, welche zu einem messbaren Effekt führten. So werden jetzt schon bei einigen Medikamenten die CYP Induktion oder Inhibierung auf dem Beipackzettel vermerkt. Auch bei den Polymorphismen wurden zahlreiche klinische Effekte beschrieben. Interessanterweise nicht nur im Zusammenhang mit einem veränderten Metabolismus von Medikamenten, sondern es konnten auch einige Neoplasien mit CYP Polymorphismen in Verbindung gebracht werden [39]. Welchen Einfluss die Diversität der CYPs im menschlichen Körper auf die Pharmakotherapie hat, sollte Hintergrund weiterer Forschung sein. Es könnte die Hypothese aufgestellt werden, dass die Bioverfügbarkeit eines Medikamentes in Geweben unterschiedlich ist. Dies wäre auch ein interessanter Ansatz für eine gezielte Pharmakotherapie: zum Beispiel wenn ein Prodrug nur durch ein im Zielgewebe hoch exprimierte CYP-Isoform aktiviert würde.

Die Untersuchung der Chemotherapieschemata und der CYP-Polymorphismen bilden die Grundlage einer retrospektiven Untersuchung von Chemotherapiekomplikationen in der Kinderonkologie. Unter anderem ist es das Ziel, einen neuen DNA-Chip zu etablieren. Ein sogenannter DNA-Chip erlaubt die parallele Untersuchung von mehreren Polymorphismen, indem Oligonukleotide (Sonden), die komplementär zu den gesuchten Polymorphismen sind, auf eine Trägerplatte aufgetragen werden. Bei Vorhandensein eines Polymorphismus bindet dieser DNA-Abschnitt des Patienten an die Sonde und wird mit Hilfe eines Fluoreszenzfarbstoffes detektierbar. Eine erfolgreiche Initiierung dieser Untersuchungsmethode könnte relevante Polymorphismen aufdecken und eine individuelle Anpassung der Pharmakotherapie erlauben.

Diese Arbeit soll einen Teil zur personalisierten Medizin beitragen und Startpunkt weiterer Forschung sein. Wissenschaftlern kann sie bei der Suche nach Informationen zur Biotransformation und deren Beeinflussungen viel Zeit ersparen und einen Überblick in Sekunden verschaffen. Ebenso soll die Datenbank eine Möglichkeit bieten, die Zahl der Nebenwirkungen und Todesfälle bei Polypharmazie zu senken und damit ein Werkzeug darstellen, die vielen Wechselwirkungen zu bewältigen.

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2 Anteilserklärung an den erfolgten Publikationen

Der Promovend Michael Hoffmann hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1

Michael F. Hoffmann, Sarah C. Preissner, Janette Nickel, Mathias Dunkel, Robert Preissner, Saskia Preissner
The Transformer database: biotransformation of xenobiotics
Nucleic Acids Research, 2014
Anteil: 40%

Beitrag im Einzelnen: Anteil bei der Entwicklung des Konzeptes, Zusammenstellung der Schlagwörterlisten für das Textmining, manuelle Überprüfung der Textminingergebnisse, manuelle Recherche fehlender Daten, Datenbankaufbau und Erstellung der Website, Verfassen der Publikation, Reviewprozess.

Publikation 2

Sarah C. Preissner, **Michael F. Hoffmann**, Robert Preissner, Mathias Dunkel, Andreas Gewiess, Saskia Preissner
Polymorphic Cytochrome P450 Enzymes (CYPs) and Their Role in Personalized Therapy
PLOS ONE, 2013
Anteil: 40%

Beitrag im Einzelnen: Anteil bei der Entwicklung des Konzeptes, Erstellen der Textminingbedingungen, manuelle Überprüfung der Textminingergebnisse, manuelle Suche nach fehlenden Daten, Expressionsanalyse von Cytochrom P450, Verfassen der Publikation, Reviewprozess.

Publikation 3

Saskia Preissner, Mathias Dunkel, **Michael F. Hoffmann**, Sarah C. Preissner, Nikolai Genov, Wen Wei Rong, Robert Preissner, Karlheinz Seeger
Drug Cocktail Optimization in Chemotherapy of Cancer
PLOS ONE, 2012
Anteil: 30%

Beitrag im Einzelnen: Anteil bei der Entwicklung des Konzeptes, Erstellen der Textminingbedingungen und Medikamentenlisten, manuelle Überprüfung der Textminingergebnisse, Verfassen der Publikation, Reviewprozess.

Unterschrift des Doktoranden Michael Hoffmann

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3 Druckexemplare der ausgewählten Publikationen

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The Transformer database: biotransformation of xenobiotics

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ABSTRACT

As the number of prescribed drugs is constantly rising, drug–drug interactions are an important issue. The simultaneous administration of several drugs can cause severe adverse effects based on interactions with the same metabolizing enzyme(s). The Transformer database (http://bioinformatics.charite.de/transformer) contains integrated information on the three phases of biotransformation (modification, conjugation and excretion) of 3000 drugs and >350 relevant food ingredients (e.g. grapefruit juice) and herbs, which are catalyzed by 400 proteins. A total of 100 000 interactions were found through text mining and manual validation. The 3D structures of 200 relevant proteins are included. The database enables users to search for drugs with a visual display of known interactions with phase I (Cytochrome P450) and phase II enzymes, transporters, food and herbs. For each interaction, PubMed references are given. To detect mutual impairments of drugs, the drug-cocktail tool displays interactions between selected drugs. By choosing the indication for a drug, the tool offers suggestions for alternative medications to avoid metabolic conflicts. Drug interactions can also be visualized in an interactive network view. Additionally, prodrugs, including their mechanisms of activation, and further information on enzymes of biotransformation, including 3D models, can be viewed.

INTRODUCTION

The number of prescribed drugs is rising (1). A study revealed that 87.1% of people >50 years of age take at least one drug per day, and 43.3% take >5 (2). Polypharmacy, which is defined as the regular use of five or more drugs, leads to an increased risk of adverse drug reactions (ADRs). The frequency of ADR is associated with the number of drugs prescribed (3). Among hospitalized patients, ADRs have an incidence of 6.7% and are the fifth commonest cause of death (4). One possible cause for ADR might be the individual variance of drug metabolism (5), and age-related changes make elderly patients more sensitive to ADRs (6). The information is widely scattered over the scientific literature. A knowledge base of xenobiotic metabolism and the effect of polymorphisms could prevent ADR and cases of death.

Xenobiotic metabolism and detoxification (especially for drugs) are separated into three different phases of reaction. Only a few xenobiotics are excreted unchanged in urine or feces without any metabolic degradation.

Phase I and phase II reactions convert compounds to more water-soluble and often less active derivatives to increase excretion. Thereby, phase I reflects the production of reactive groups through oxidation and is primarily managed by the Cytochrome P450 family (CYP) of enzymes (7). Subsequently, the reactive groups are used to conjugate small polar molecules (phase II) to increase the polarity. Six enzyme families that provide the detoxification and excretion of xenobiotics mainly realize the conjugation (8).

Transporters (phase III) play a crucial role in pharmacokinetics by enabling the migration of hydrophilic molecules, which cannot penetrate cellular membranes. Kell *et al*. showed that the majority of drugs enter cells through at least one transporter (9). Those proteins form a transmembrane channel lined with hydrophilic amino acid side chains spanning the lipid bilayer (10). Two major protein superfamilies are known: 49 ATP-binding cassette transporters (ABC) (11) and 362 solute carriers (SLC) (12). These are important for absorption, distribution and

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as Joint First Authors.

excretion of drugs (13) and are involved in a broad range of physiological processes (10).

Eukaryotic ABC transporters are predominantly exporters, which require energy released by ATP hydrolysis. One problem is multidrug resistance, which is caused by active transporters. Unfortunately, 40% of human tumors develop resistance to chemotherapeutics by overexpressing ABC proteins (14). The SLC transporters facilitate passive diffusion along the concentration gradient or use concentration gradients from other substrates as a symporter or antiporter (12).

Another issue related to drug metabolism and ADR is prodrugs. Prodrugs have to be converted to active drugs by metabolic conversion (15). In general, prodrugs are non-toxic and need to have their chemical structure changed to enable their inherent medical capability. However, problems in conversion can also lead to undesired side effects. For example, the antihistamine terfenadine is a potent hERG blocker as a prodrug and a slow conversion can cause cardiac toxicity (16).

Prodrugs can be activated by photo irradiation (17), a change in pH (18) and enzymes, such as esterases or CYPs (19,20). Many prodrugs are activated by hydrolysis with the aid of esterases or phosphatases. Thereby, gastric intestinal tolerance and pharmacokinetics can be improved, but the targeting of drugs to specific cells or tissues cannot. The activation of prodrugs by CYPs might be a better approach (21).

Not only can drugs participate in the alteration of drug metabolism but food and herbs also have a proven influence; e.g. furanocoumarins in grapefruit inhibit intestinal CYP3A4 and organic anion-transporting polypeptides 1A2 (22,23).

More than 350 ingredients in food and drink, such as broccoli, alcohol and char-grilled meat, as well as herbal medicine, such as St John's wort, are known to alter drug responses.

A comprehensive resource that combines scientific information on phase I and phase II enzymes, transporter enzymes, prodrugs, food and herbs could help to improve research in this field and prevent ADR.

MATERIALS AND METHODS

Text mining

We created a text mining approach using semantic web standards. To develop a specialized text mining pipeline, we first downloaded Medline/PubMed data from the NCBI FTP site in xml-format. Using the search engine library Apache Lucene (http://lucene.apache.org) and a tool kit for processing text with computational linguistics (http://alias-i.com/lingpipe), the data was indexed. The search engine comprises comprehensive lists of chemical compounds and drug names (24), metabolic enzymes (25) and transporters (26), including their various synonyms. Additionally, we added a list of common interaction terms, such as 'activate', 'inhibit', and 'metabolize'. The search engine, written in Java, dynamically queries the indexed data and produces a structured query language (SQL) file containing the text mining hits. A query example is:

```
(DrugSynonym [TI] AND TransformerSynonym
  [TI]) OR
(DrugSynonym [abstract] AND TransformerSynonym
  [abstract]) OR
(DrugSynonym [abstract] AND InteractionTerm AND
  TransformerSynonym [abstract])
```

The positional distance between the different terms had to be restricted to reduce false-positive hits, when terms occurred far from each other in the abstract. The 22 500 records found were scored as rule-based. Duplicates were removed and a team of scientists manually processed 12 427 articles found in PubMed. Further details about the text mining approach can be found on the Web site in the frequently asked questions (FAQs) section.

Database

The database was designed as a relational database on a MySQL server. To allow chemical functionality, such as handling chemical data within MySQL, the MyChem package was included.

Information about ~3000 CYP drug interactions and 2000 polymorphisms were extracted from the SuperCYP database (27). SuperCYP is a database with a focus on human CYPs. However, there are many other important enzymes in the metabolism of xenobiotics, such as transporters or phase II enzymes.

DATABASE FEATURES

Over 100 000 interactions were revealed. In the 12 427 articles found in PubMed, 769 drugs were attributed to those phase II enzymes that are involved in drug metabolism. Text mining was also performed for prodrugs, transporters and food. We found 125 prodrugs described in 890 PubMed articles together with their mechanism of activation, accompanying enzymes, chemical structure and identification numbers. Furthermore, ~500 drug-transporters and 150 food interactions were identified.

Additionally, ~200 3D structures were collected for transporters, CYPs and phase II enzymes.

The database includes four main functionalities. To provide an overview of these, a comprehensive FAQs section was created, which is helpful for first-time users. Depending on the user's interest or needs (e.g. clinicians, researchers), different ways to browse the data were enabled.

Prodrugs

Prodrugs can be identified directly by entering the name, PubChemID, CAS number or ATC code, as well as by choosing a mechanism of activation, such as ring opening or carboxylation.

Drugs

To view the metabolism of particular drugs, users can search directly by entering the name, PubChemID or

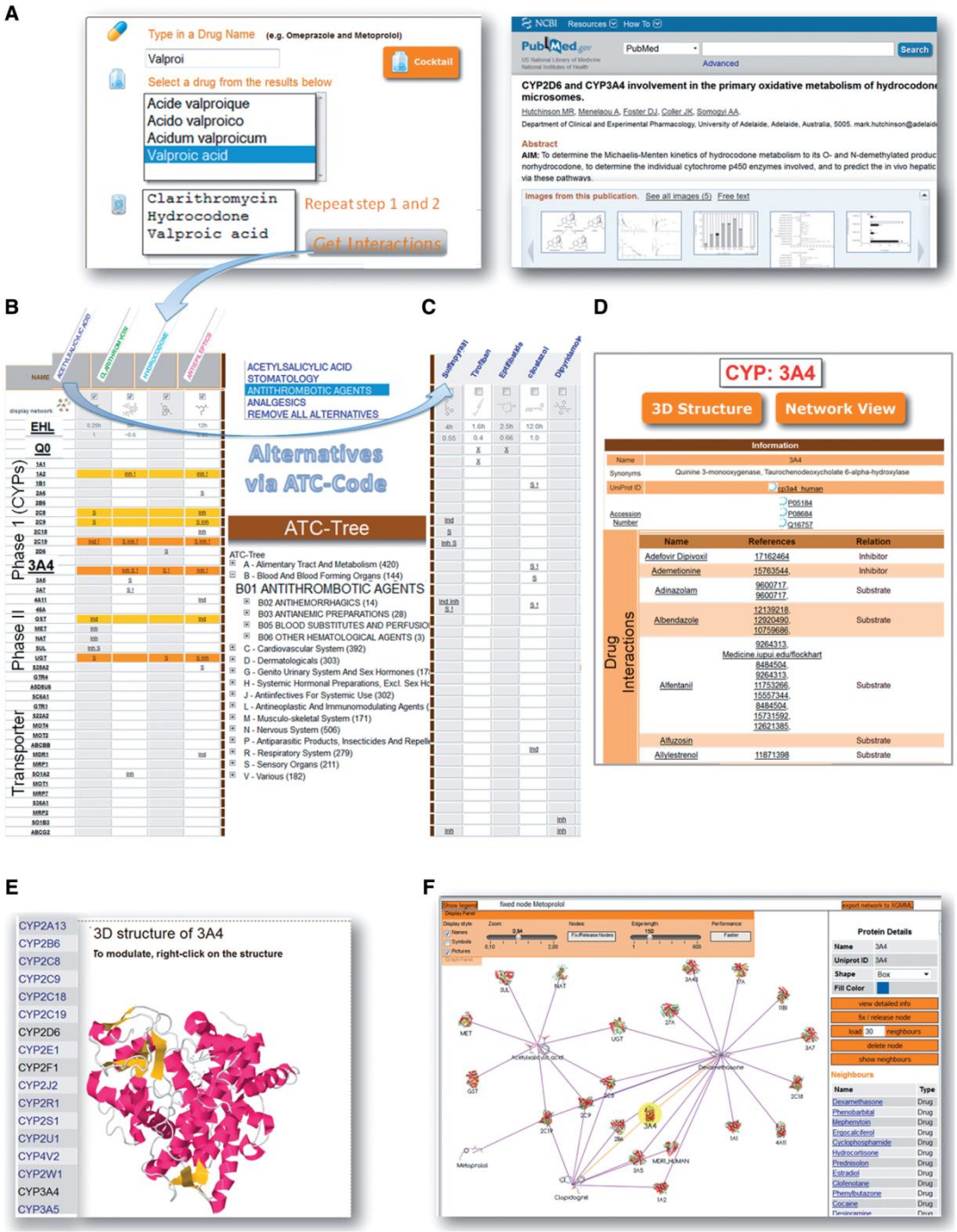

Figure 1. Functionalities of the 'Transformer database'. (**A**) Composition of a drug-cocktail. An example of a PubMed reference is shown. PubMed references can be viewed by clicking on 'S', 'Inh' or 'Ind' in the result table. (**B**) Clicking on 'Get Interactions' leads to a result table, which shows the interactions between the drugs. (**C**) By choosing the indications of the drugs via ATC-code the user receives specific alternatives. (**D**) By clicking on an enzyme (e.g. CYP3A4), detailed information on the enzyme, including drug interactions, are shown. (**E**) The 3D structures of all enzymes can be viewed (e.g. CYP3A4). (**F**) Network views are provided for each enzyme and compound.

CAS number. Based on the WHO classification system, which classifies drugs into different groups according to Anatomical site of action, Therapeutical effect and Chemical structure (ATC), a tree with all of the drugs contained in the database can be viewed in their ATC group.

Cocktail

This tool enables users to see drug interactions of an individually composed drug cocktail (Figure 1A). If >1 drug interacts with the same enzyme, lines of the interaction table are shown in yellow, orange, red and dark red (Figure 1B). In the header of each column, the indication for the drugs can be chosen and the database will provide alternative drugs that are metabolized by different enzymes (Figure 1C). PubMed references are available by clicking on the interaction. Additionally, food interactions, as well as elimination half-life (EHL) times and Q_0 values are displayed. A Q_0 value (extrarenal excretion) of <0.3 is shown in green because those drugs are, to a large extent, excreted in the unchanged form. Clicking 'Display network' presents these interactions in a network view based on Cobweb (28).

Biotransformation

To find drugs that are metabolized by specific phase I, phase II or transporter enzymes, users can perform a search by clicking on 'Biotransformation'. This page provides (homology modeled) 3D structures of all enzymes (Figure 1E). Furthermore, a list of interacting drugs can be viewed in a table (Figure 1D) or in a network view (Figure 1F).

DATABASE USAGE

The following case illustrates the need to detect interactions with the help of the Transformer database. A five-year-old child died from a fatal opioid toxicity. She was inadvertently administered a high dose of hydrocodone (an antitussive drug) while suffering from a cold. Additionally, she was administered clarithromycin for an ear infection and valproic acid for seizures. The postmortem blood screen revealed an excessively high-hydrocodone level and, in contrast, barely measurable hydromorphone (biotransformation metabolite of hydrocodone) concentration (29). Hydrocodone is metabolized by CYP2D6, CYP3A4 and afterwards by UGT. There were three reasons for the low metabolism rate:

(1) CYP-polymorphism: the child was found to be a CYP2D6 poor metabolizer,
(2) inhibition of CYP3A4 by clarithromycin and
(3) inhibition of UGT by valproic acid.

Figure 1B shows the Transformer database results for this drug combination. All interactions described in this case are displayed and colored because of enzyme overload. Nevertheless, parts of the available information of drug–enzyme interactions are experimental data and offer no evidence for drug interactions in humans and clinical work, although Q_0 and elimination half-life times could be relevant. The Transformer database, however, provides a platform for detecting mutual drug impairments and could help to appraise the drug response. The database is a comprehensive resource on drug enzyme/transporter interactions and could be a sound starting-point for further research.

The database will be updated yearly to add new drugs/compounds and interactions.

AVAILABILITY

The Transformer database is publicly available via http://bioinformatics.charite.de/transformer and should be used under the terms of the Creative Commons Attribution-Noncommercial-Share Alike 3.0 License.

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PLOS ONE

Polymorphic Cytochrome P450 Enzymes (CYPs) and Their Role in Personalized Therapy

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Abstract

The cytochrome P450 (CYP) enzymes are major players in drug metabolism. More than 2,000 mutations have been described, and certain single nucleotide polymorphisms (SNPs) have been shown to have a large impact on CYP activity. Therefore, CYPs play an important role in inter-individual drug response and their genetic variability should be factored into personalized medicine. To identify the most relevant polymorphisms in human CYPs, a text mining approach was used. We investigated their frequencies in different ethnic groups, the number of drugs that are metabolized by each CYP, the impact of CYP SNPs, as well as CYP expression patterns in different tissues. The most important polymorphic CYPs were found to be 1A2, 2D6, 2C9 and 2C19. Thirty-four common allele variants in Caucasians led to altered enzyme activity. To compare the relevant Caucasian SNPs with those of other ethnicities a search in 1,000 individual genomes was undertaken. We found 199 non-synonymous SNPs with frequencies over one percent in the 1,000 genomes, many of them not described so far. With knowledge of frequent mutations and their impact on CYP activities, it may be possible to predict patient response to certain drugs, as well as adverse side effects. With improved availability of genotyping, our data may provide a resource for an understanding of the effects of specific SNPs in CYPs, enabling the selection of a more personalized treatment regimen.

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Introduction

Inter-individual variability of drug response and drug clearance is a complex and common problem in clinical practice [1]. Overlapping substrate specificity of enzymes, a multitude of single nucleotide polymorphisms (SNPs) [2] and variations between ethnic groups [3] make prediction of phenotypic drug response difficult. To avoid treatment failure and unnecessary toxicity, tailoring dosages and drug-cocktails for each individual is essential [4].

Differences in drug response can be attributed to variability in DNA sequences of specific genes which's products are crucial for drug metabolism. For instance, SNPs in phase 1 enzymes, such as cytochrome P450 oxidases (CYPs) [3], phase 2 enzymes, such as Uridine 5'-diphospho-glucuronosyltransferase (UGTs) [5], and absorptive and efflux transporters, such as ATP-binding cassette transporters (ABC-transporters) [4], have been previously reported. Characterization of these enzymes and the effects of minor allele variants on the metabolism of specific drugs have been described in the literature and have recently been compiled by our group into a comprehensive database called SuperCYP [6]. Phase I reactions include oxidation, reduction, hydrolysis and cyclization. Using oxygen and NADPH as a co-substrate, CYPs are the major enzymes responsible for catalyzing such reactions [7] and account for approximately 75% of total drug metabolism [8].

The Human Genome Project identified 57 human CYPs, which were classified into 18 families and 43 subfamilies based on sequence similarity [9]. CYP families 1, 2 and 3 are responsible for metabolism of drugs, xenobiotics and certain endogenous molecules [3] and hence are of particular relevance to this current study. Most CYPs metabolize more than one drug. Similarly, a drug is often metabolized by multiple CYPs. Drugs can also inhibit or induce CYP activity, either by directly interacting with the enzyme or altering its

expression. Characterization of these interactions is important to determine and predict compatible drug combinations [10]. Human CYPs are primarily membrane-associated proteins [11] that are ubiquitously expressed in most tissues. Highest expressions are generally found in liver tissue, but the distribution of particular CYPs varies [12], which indicates that the actual efficiency of a drug is likely to depend on CYP expression in the target tissue. There are significant inter-individual differences in enzyme activity leading to distinct phenotypes. For example the most frequent phenotype of CYP 2D6 is the extensive-metabolizer (78.8%), followed by intermediate- (12.1%), poor (7.6%) and ultra-rapid metabolizers (1.5%) [13].

In addition to drug catabolism, many CYPs are responsible for activation of prodrugs, such as cancer therapeutics [14] and antipsychotics [15]. Prodrugs are pharmacologically inactive compounds that require activation via metabolic conversion [16], allowing control of where, when and how much drug activity occurs [17]. This is particularly important for chemotherapeutic drugs, where the active drug ideally only acts on tumor cells in order to reduce toxic side effects [18]. Prodrugs can be activated by photo irradiation [19], change in pH [20] or enzymatically [21], for instance by CYPs [22]. Polymorphisms in CYPs can result in ineffective or aberrant activation of prodrugs [22], which can lead to toxicity [4]. Fortunately, advances in genetic research have made genotyping of a large number of patients possible, leading to identification of SNPs that alter expression or activity of drug metabolizing enzymes [3]. In this study we set out to determine the most frequent CYP polymorphisms having the highest impact on drug metabolism in Caucasians. This knowledge could facilitate the development of tests for efficient genotyping of patients thus leading to a better and more personalized treatment.

Methods

Text mining

Information on drug metabolism can be found in more than 100,000 PubMed articles, yet limited data is available regarding the frequencies of SNPs in human CYPs. To identify relevant articles, a specific search tool was developed for text mining literature using Apache Lucene™ (http://lucenenet.apache.org) as a search engine library and LingPipe (http://alias-i.com/lingpipe). Figure 1 summarizes the different methods used for the textmining approach. Complete Medline/PubMed data were downloaded from the NCBI FTP site in xml-format and then indexed. The indexed data was dynamically queried by a search engine written in Java that outputs an sql-file with the text mining hits, which served afterwards for manual validation. The search engine comprises several lists of synonyms for identifying entities, such as chemical compounds, biological targets, genes, cell types and polymorphisms, as well as interaction-related entities. If available, information on CYP polymorphism was extracted from the literature. Definitions and synonyms are included from UMLS® Metathesaurus®, that contains millions of biomedical and health related concepts, their synonymous names, and their relationships. As an example, the query for CYP2C19 was like:

(Abstract: CYP2C19** OR Title: CYP2C19**) AND (Abstract: population OR Title: population) AND (Abstract: effect OR Title: effect) AND (Abstract: frequenc* OR Title: frequenc*).

The term 'CYP2C19' was replaced through each human CYP and synonyms, as well as different ethnicity and outcome terms were used for 'population' and 'effect'. The positional distance between the different terms had to be restricted to reduce false positive hits, when terms occurred far from each other in the abstract. The records found were scored rule-based. The rules employed order, redundancy, distance, topic segmentation and sentence breaking for boundaries. For example, a distance $\leqq 7$ between the CYP and the ethnicity and $\leqq 6$ between the frequency and the CYP was given a score of 100. Greater distances and negative interaction words resulted in lower scores. Duplicates were removed and a team of scientists manually processed 1,037 papers found in PubMed for relevance to polymorphisms and their frequency in Caucasian populations. The team consisted of three medical scientists, with three years experience in validation of text mining results. During this time, they reviewed over 10,000 abstracts with the focus on CYPs. A weekly meeting took place to ensure and raise the quality of text mining and to discuss problems. The aim was to achieve a coherent review operation. The text mining validation tool is shown in Figure 2. CYP polymorphisms that occurred with a frequency of more than one percent in the Caucasian population were included in this study.

Localization of SNPs in a 3D CYP model

The evolutionary conservation taken from a multiple sequence alignment of CYPs was projected onto the 3D structure using CYP 2D6 as template (PDB ID: 3TDA). Frequent SNPs in the four most polymorphic CYPs (1A2, 2C9, 2C19 and 2D6) were labeled in the 3D model. The number of mutations was used to determine the thickness of the ribbon (Figure 3).

CYP SNPs and 1,000 Genomes

The 1,000 Genomes Project (www.1000genomes.org) is an international initiative designed to provide full genomic sequence information from an ethnically diverse population [23]. CYP SNPs in 1,092 individuals were extracted using the online data slicer from the 1,000 Genomes Project (http://browser.1000genomes.org). Frequency analysis focused on non-synonymous coding SNPs with a prevalence of one percent or higher in all genomes regardless of ethnicity. The search included the main 29 CYP alleles from "The Human Cytochrome P450 (CYP) Allele Nomenclature Database" (http://www.cypalleles.ki.se/) [24]. In addition, 16 CYP alleles not listed in the CYP allele database due to very heterogeneous distributions were included. The 1,000 Genomes Database includes SNP effect predictions on CYPs, calculated by PolyPhen [25], which predicts possible functional alterations in human proteins after amino acid substitution based on physical and comparative considerations [26].

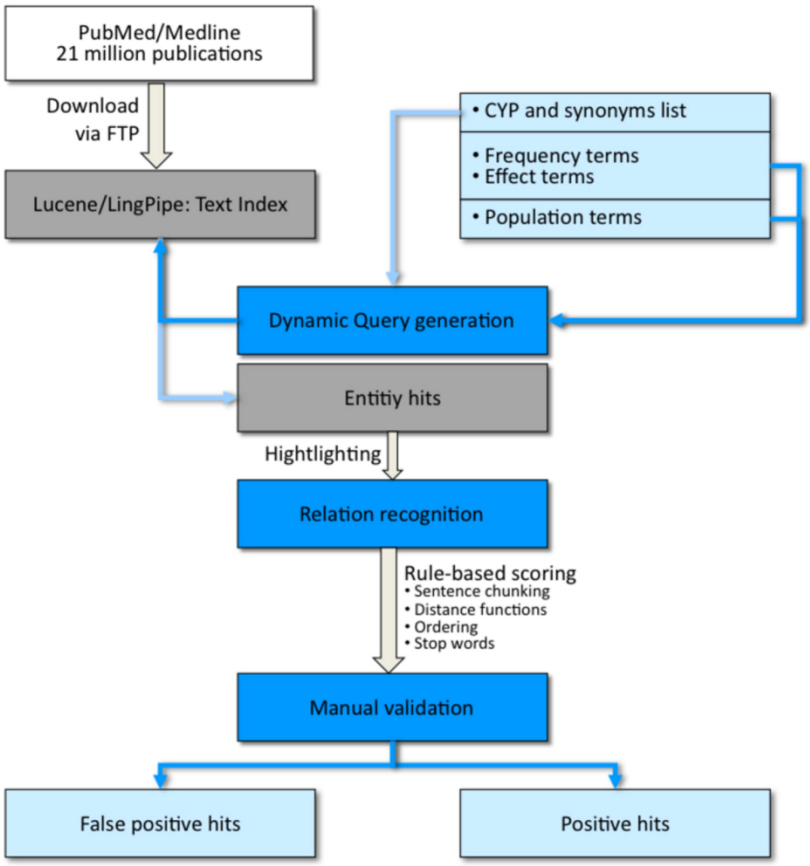

Figure 1. Flow-chart of the methods used for the text mining approach.
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Expression data

Affymetrix data was used to compare human CYP mRNA expression in 41 different types of tissue, further subcategorized into different regions of an organ, yielding a total of 65 tissue types. The series of datasets obtained from GEO (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) were originally generated from 10 post-mortem donors (5 females and 5 males), and represent normal human tissues (Series GSE3526) [27]. The 84 probe sets, which measure the expression level of CYPs were normalized and assigned to 40 types of CYPs. To display differences in expression, a heat-map was generated using Genesis software [28]. Relative expression was calculated as the intensity of the gene in the region minus the mean intensity of the gene in all regions then divided by the standard deviation. This heat-map served as data source for the CYP body map in which only two-fold decreased or increased values were considered.

Our work would not have been possible without the publicly available datasets mentioned above. We are grateful and honor the work of involved research groups.

Results

Frequencies of SNPs in CYPs

Analysis of the SNPs identified by text mining, showed that SNPs predominantly occurred in 3 polymorphic CYPs (2D6, 2A6 and 2B6) regardless of ethnic group. Only frequencies of known nucleotide changes were assessed to identify the extent of SNPs in CYPs. Figure 4 displays 9 CYPs, including 2D6 (114 SNPs), 2A6 (68 SNPs) and 2B6 (57 SNPs), which showed the highest number of SNPs. For other CYPs, the number of known SNPs was less than 22. CYP 2D6 is a major polymorphic CYP and, as expected, was the greatest contributor of polymorphic alleles in Caucasians.

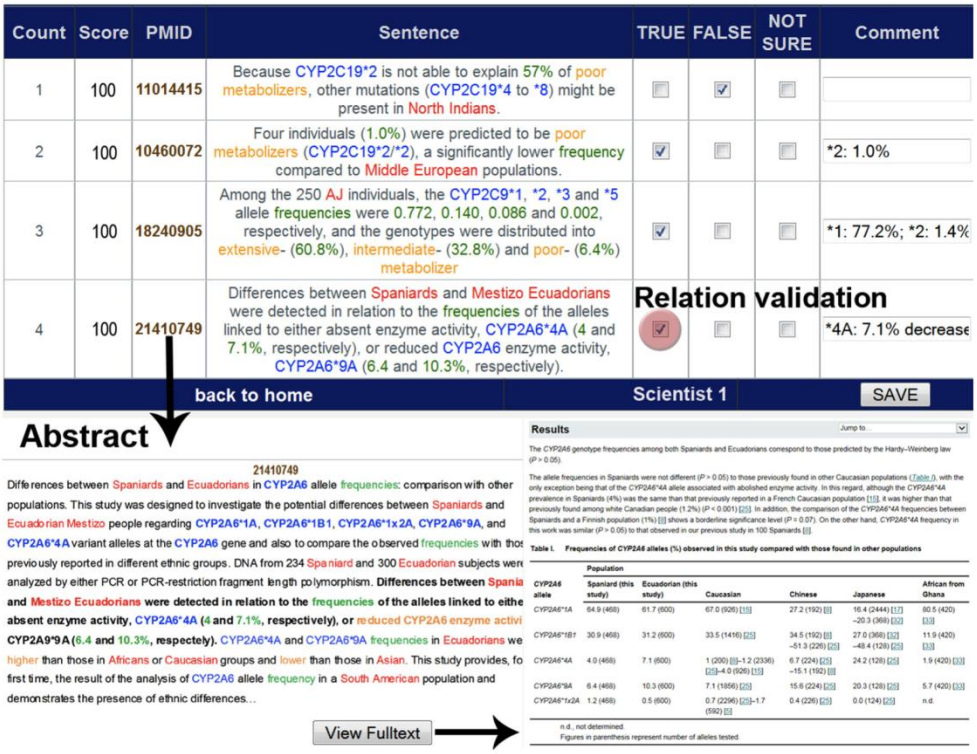

Figure 2. Text mining validation tool. The table shows the text mining validation tool with columns for score, PubMedID, relation sentence and checkboxes for the validation. The SNPs are highlighted in blue, frequencies in green, effects in orange and the ethnicities in red. 'Scientist 1' reads the abstract and, if necessary, has access to full text. Afterwards, the relation has to be validated as 'true', 'false' or 'not sure'. If the relation is 'true', the relation is copied into the 'comment' field. These relations are copied into a new sql-file. If 'Scientist 1' activates the 'not sure' field, the relation has to be validated again by another scientist.

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Allelic frequency in CYPs of Caucasians

The PubMed search yielded articles on 34 different CYP alleles with an occurrence greater than one percent in the Caucasian population (Table 1), which may be indicative of altered substrate metabolism. CYP 2D6 and 2B6 possessed the largest number of alleles with a known impact on metabolism in Caucasian population (11 and 6, respectively). Maximum allele frequencies in CYP 2D6 varied from 20.7% to 32.4%. When considering all 34 alleles, the most frequent alleles were CYP 3A5*3C at 81.3% (decreased enzyme activity) followed by CYP 1A2*1F at 33.3% (increased enzyme activity). Furthermore, the major alleles leading to increased metabolism were CYP 2A6*1B (30.0%), 3A4*1B (17.0%), 1A1*2A (19.0%) and 2C19*17 (18.0%). In contrast, decreased metabolism was attributed to 2D6*2A (32.4%), 2D6*4 (20.7%) and 2C9*2 (16.0%). Carrying the 2A6*4 allele (1.0%) leads to an inactive enzyme with no detectable substrate metabolism.

Comparison among different ethnic groups revealed that frequencies differed considerably and displayed a heterogeneous distribution of CYP alleles. For instance, in Asian and African populations, CYP2A6*2 possessed a frequency of 28.0% and 62.0%, respectively, whereas a frequency of 8.0% was observed in Caucasians. A more detailed table with additional information on CYP SNPs in Caucasians and other ethnic groups is available in Table S1.

Major drug metabolizing CYPs

Not all 57 human CYPs are involved in drug metabolism. The primary CYPs responsible for drug metabolism were determined by first ranking the CYPs according to the total number of drug substrates (Figure 5). Twelve CYPs accounted for 93.0% of drug metabolism, regarding to the entire number of 1.839 known drug-metabolizing-reactions in the SuperCYP database. CYP 1A2, 2D6, 2C9 and 2C19 were responsible for nearly 40.0% of drug metabolism and including CYP 3A4 even for 60.0%.

Since the described four CYPs are highly polymorphic and commonly occur in Caucasians, further detailed analyses were

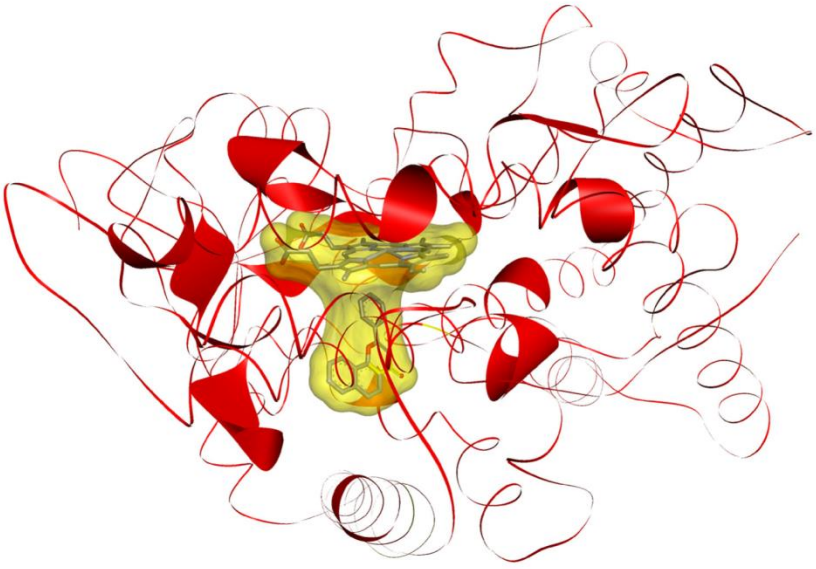

Figure 3. 3D structure of a cytochrome with heme and a ligand with localization of frequent SNPs. Frequent SNPs in the four most polymorphic CYPs (1A2, 2C9, 2C19 and 2D6) were labeled in this 3D model of CYP 2D6 (PDB ID: 3TDA). Therefore, the ribbon was enlarged in the appropriate positions. The binding-side (transparent, orange colored surface) contains an iron ion and a porphyrine ring (heme). Frequent mutations of CYPs at the binding-side occurred at the following positions: 67, 89, 107, 117, 118, 120, 125, 132, 151, 201, 227, 261, 325, 377, 382, 386, 410, 454, 456, 469 and 470.
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restricted to these four CYPs. On the overall CYP system, it is expected that these four CYPs would have the greatest impact on inter-individual variability of drug response. Although CYP 2A6 and 2B6 possess various relevant alleles in Caucasians, they do not cover a large range of drug interactions (51 and 74 substrates, respectively).

All SNPs in the four most polymorphic CYPs (1A2, 2C9, 2C19 and 2D6) influenced enzymatic activity due to localization in the substrate-binding cavity as shown in Figure 3.

Expression data

Because of high CYP expression levels in some tissues, an impact of CYP isoforms in particular tissues can be deduced. The work of Nishimura and colleagues demonstrated differences in CYP mRNA expression in various human tissues. For example, CYP 2F1, 4B1, 4F8, 11S, 11A, 11B1, 11B2, 19 and 24 are not expressed in the liver [29]. The current study confirmed these results and extended the findings, which are shown in Figure 6. Nishimura analyzed mRNA levels of 30 CYP isoforms in 11 tissue types. Similarly, the current study investigated the expression of 40 CYP isoforms in 41 tissue types. The liver was considered separately in the analysis in order to identify the differences between the other tissues. In 21 different tissues, a heterogeneous distribution of CYPs was observed. For instance, 39 different CYP isoforms showed higher mRNA expression in at least one or more tissue types. Significant differences were observed in the adrenal gland

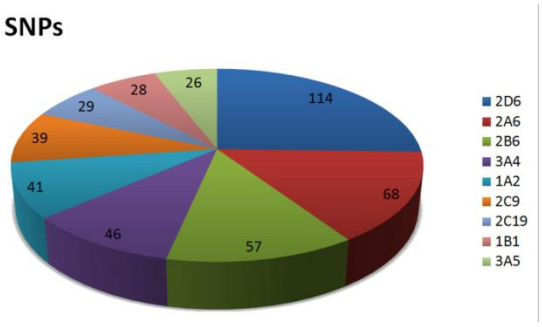

Figure 4. Number of known SNPs per CYP. The pie chart illustrates the number of known SNPs in different CYPs.
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cortex, which possessed 6-fold higher expression of CYP 11A1, 11B1 and 11B2 (compared to the mean expression). Interestingly, no other tissue showed high levels of expression of these three CYPs. Large differential expression compared to other tissues was also observed in the kidneys, where a 6-fold increase in CYP 4A22, a 5-fold increase in CYP 8B1 and 4-fold increases in 4V2, 4F2, 4A11 and 2B6 were noted. In addition, 5-fold higher expression of CYP 2C8 was found in lung, CYP 4F8 in prostate, 4F3 in bone, 2F1 in bronchial tubes and 2C8 in

Table 1. CYP SNP frequencies in Caucasians.

CYP	Allele	Amino Acid	Caucasian (%)	Enzyme Activity	Test Drug	References (PMID)
1A1	*2A	I462V	19.0	increase	17β-estradiol	19514967
1A2	*1F	none	33.3	higher inducibility	Omeprazole	12534642 / 22299824
	*1D	none	4.82	decrease	Clozapine	12534642 / 20797314
2C9	*2	R144C	19.0% *1/*2 1.6% *2/*2 1.8 % *2/*3	decrease	Warfarin	15284536
	*3	I359L	9.0	decrease	Tolbutamide	11678789
2C19	*2	Splicing I331V defect	16.0	decrease	Clopidogrel	10460072
	*17	I331V	18.0	increase	Omeprazole	21247447
2D6	*3	N166D; 259 Frameshift	2.04	decrease	Debrisoquine	9012401
	*4	P34S; L91M; H94R; Splicing defect; S486T	20.7	decrease	Dextromethorphan	9012401
	*4D	P34S; Splicing defect; S486T	3.4	decrease	Bufuralol	11266079
	*4L	P34S; Splicing defect; S486T	4.5	decrease	Bufuralol	11266079
	*5	CYP2D6 deleted	4.1	no enzyme		9511177
	*6	118Frameshift	1.3	nonfunctional		9511177
	*7	H324P	1.0	decrease	Sparteine	9089660
	*9	K281del	2.0	decrease	Sparteine	9511177
	*10	P34S; S486T	8.0	decrease	Metoprolol	9511177 / 11505219
	*41	R296C; Splicing defect; S486T	8.0	decrease (expression)		15289790
2A6	*12	10 aa substitutions	2.9	decreased (expression)		16041240
	*1B	none	32.6	increase	Caffeine	22850738
	*2	L160H	2.3	decrease	Nicotine	11259354
	*4	CYP2A6 deleted	1.0	no enzyme		11259354
	*9	(TATA box)	7.1	decreased	Nicotine	15475735
3A4	*17	F189S	2.0	decrease	Testosterone	11714865
	*1B	none	17.0	increase (transcription)	Tacrolimus	12692107
	*2	S222P	2.7	decrease	Nifedipine	10668853
3A5	*3C	Splicing defect	81.3	decrease	Sirolimus	17162466
	*3k / *10	Splicing defect; F446S	2.0	decrease	Nifedipine	12893984
3A7	*2	T409R	8.0	increase	Dehydroepiandrosterone	15903124
2B6	*2	R22C	5.3	increase	Artemether	21746968 / 12242601
	*5	R487C	14.0	decrease	Nirvanol	11470993
	*4	K262R	5.0	increase	Bupropion	14515060
	*6	Q172H; K262R	25.2	decrease (expression)	Cyclophosphamide	14515060
	*7	Q172H; K262R; R487C	3.0	decrease	7-ethoxy-4-trifluoromethylcoumarin	12242601 / 14551287
	*22	none	3.0	increase (transcription)		15722458

Polymorphisms that are relevant for Caucasians are shown here with CYP, allele, amino acid and frequencies and their effect on enzyme activity. The test drug and the PubMed ID complete the table. A more detailed table including gene information and frequencies in other ethnic groups can be found in Table S1.

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stomach. Furthermore, CYP 2C18 showed a high level distribution restricted to the oral cavity, pharynx and esophagus. Two-fold lower expression was detected for CYP 2A1 in the esophagus, 2A7 in the prostate, as well as 2C9 and 2D6 in the spleen.

CYP SNPs and 1,000 Genomes

The current study identified 199 non-synonymous coding SNPs with frequencies greater than one percent (Table S2). Compared to the "Human Cytochrome P450 Allele Nomenclature Database" (http://www.cypalleles.ki.se/), we found several SNPs in 1,000 Genomes not related to alleles defined and named in the Database. To elucidate the difference between the 'The Human Cytochrome P450 Allele Nomenclature' and 1000genome data regarding new SNPs, we examined CYP2A6 exemplary. Table 2 summarizes SNPs most likely to alter enzyme activity [25]. It displays five SNPs, which can lead to an altered enzyme activity with frequencies between 1.4 and 5.1 %. Only I471T (rs5031016) is also contained in the CYP nomenclature and reflects the CYP2A6*36 allele. New updates have to be done to map a comprehensive CYP SNP data source.

With the potential to alter drug metabolism, the 72 listed SNPs occurred in 24 CYPs. The most frequent SNPs were CYP 4A11 rs112743 (42.6%; highly expressed in kidney tissue), CYP 4F11 rs1060463 (49.5%; highly expressed in bronchus tissue) and CYP 2A7 rs3869579 (46.7%; highly

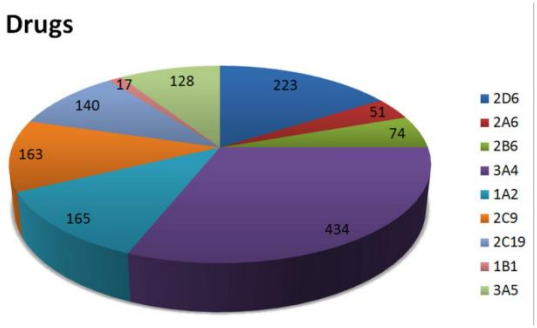

Figure 5. Number of drugs metabolized per CYP. The pie chart illustrates the number of drugs that can be metabolized by a specific CYP.
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expressed in the pituitary gland but low in the prostate gland). Our study findings show that some CYPs are not only heterogeneously expressed, but also highly polymorphic.

Discussion

Genetic diversity and polymorphisms

Mutations in a CYP gene can lead to functional alterations, such as increased or decreased activity. If a mutant allele occurs at a frequency of at least one percent in a population, it is referred to as a pharmacogenetic polymorphism. Such polymorphisms can be discovered at the genotype level and/or the phenotype level based on altered function of the enzyme [30].

Individuals in a population can be stratified according to metabolic ratios of particular CYPs, which have great clinical relevance. For example, a CYP 2D6 poor metabolizer should not be administered codeine since the drug would have no effect. Conversely, a CYP 2D6 ultra-rapid metabolizer would likely suffer side effects from a normal dosage [31,32]. CYP 2D6 is a highly polymorphic CYP with at least 70 allelic variants [33] that can be categorized into four phenotypic classes. Overall CYP 2D6 expression in liver tissue is only approximately 2%, but hundreds of drugs are metabolized by this enzyme, including opiates, beta-blockers, anti-arrhythmics, tricyclic antidepressants, SSRIs, 5-HT3-antagonists and neuroleptics [34]. About 10% of the Caucasian population have difficulties in fully metabolizing these drugs [35], leading to harmful side effects [32,36]. Therefore, personalized prescriptions will become of great importance [37].

Personalized medicine

Since 2009, the Clinical Pharmacogenetics Implementation Consortium (CPIC) provides information on how genetic test results can be used to optimize drug therapy. The guidelines center on genes or on specific drugs. For some drugs, they also provide dosing guidelines for clinicians [38].

Psychiatric drugs. As most psychiatric drugs are metabolized by highly polymorphic CYP 2D6 and CYP 2C19, psychiatrists were first to propose the idea of CYP genotyping [39–41]. Three state hospitals in Kentucky recruited 4,532 psychiatric patients for genotyping of both CYPs with the help of DNA microarray technology.

Results from the current study were consistent with previous studies of allele frequency [35], demonstrating the importance of personalized prescription given that more than one tenth of patients are not likely to respond to standard treatment and suffer unwarranted toxicity. In the study performed by de Leon and colleagues, the dosage was adapted to the guidelines of Kirchheiner [15] for antipsychotics and antidepressants. The authors propose a numeric dosage adaptation system that reflects expression of CYP 2D6 and CYP 2C19.

Cardiovascular drugs. An important area of focus is stent implantation and/or inhibition of blood clots after an acute coronary syndrome (ACS) to prevent ischemic events. Therefore, antiplatelet agents are administered before and after percutaneous coronary intervention (PCI) to reduce the risk of ischemic events. Currently, the gold standard therapy is a combination of aspirin and clopidogrel [42,43]. Unfortunately, approximately 29% of people respond poorly to clopidogrel [44] and, therefore, have an increased risk for recurrent ischemic events after PCI [45]. Several different factors were discovered to contribute to the variability in clopidogrel response, including polymorphisms, impaired absorption or bioavailability, poor compliance and pre-existing conditions (increased body mass index, diabetes mellitus, ACS) [46]. In addition, clopidogrel is a prodrug that requires activation through the CYP system. The activated metabolite inhibits the ADP P2Y12 receptor [47]. Polymorphisms causing loss of function in the CYP system are associated with poor drug response. Most notably, the CYP 2C19*2 polymorphism was shown to lead to a 30% increased risk of major adverse cardiovascular events during treatment with clopidogrel [48–51]. Furthermore, the CYP 2B6*5 and P2Y12 polymorphisms are also associated with clopidogrel resistance [52]. In contrast, an enhanced response due to increased transcriptional activity occurs with the CYP 2C19*17 polymorphism, leading to increased risk of bleeding during clopidogrel therapy [53,54].

The CYP3A4*2 allele with a frequency of 2.7 % in Caucasian leads in vitro to reduced (six fold to nine fold) intrinsic clearance for nifedipine [55]. This could have a great influence on the tolerability of patients getting this dihydropyridine calcium channel blocker. Indications for nifedipine are widely distributed, e.g. Angina pectoris, Hypertonia, Achalasia and Raynaud's phenomenon, so the application is very common. An in vivo research regarding the alteration of nifedipine metabolism in CYP3A4*2 patients should be done, to possibly prevent toxic and/or increased side effects.

Previous findings described above, emphasize the importance of CYP polymorphisms and alternatively metabolized drugs in clinical practice. Prediction of CYP activity may be helpful to assess drug response. For instance, the (13)C-pantoprazole breath test, which measures CYP 2C19 activity, can detect clopidogrel resistance [56] and support use

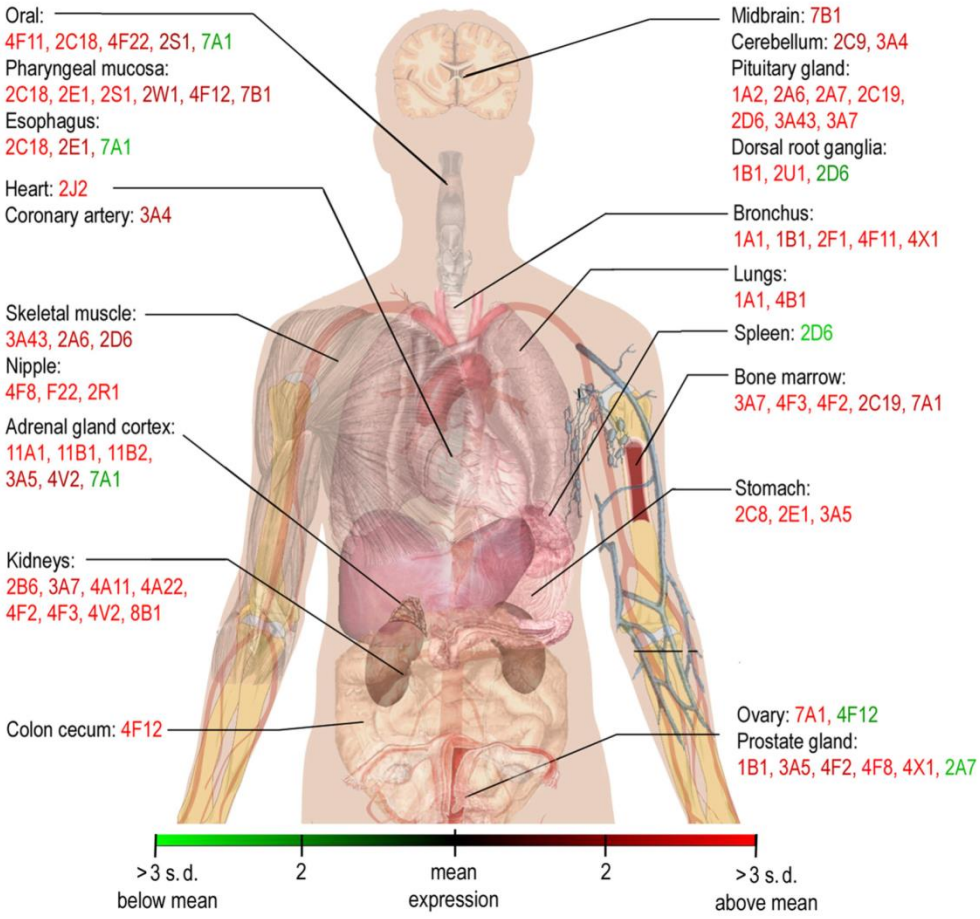

Figure 6. Body map of cytochrome P450 enzyme expression. A schematic map of the specific expression of different CYPs in human organs is presented. Expression values are relative to the mean expression in all organs. At least three-fold higher expression of a CYP in one organ is indicated by red text. At least three-fold lower expression is indicated by green text. A color spectrum for the expression values is illustrated in the provided scale. CYPs with average expression in one organ were not included.

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of suitable drug alternatives like Ticagrelor (no activation required, metabolized via CYP 3A4).

Additional observed effects

Apart from altered drug metabolism, CYP polymorphisms were also potentially associated with neoplastic growth, adverse psychological behavior and other diseases. In women, polymorphisms in CYP 1A1 seemed to increase susceptibility to genital cancers [57,58]. Conversely, the CYP 2D6*4 polymorphism has been shown to have a protective effect against breast cancer [59]. Furthermore, 2C19*2, 2D6*4, 2D6*10 and 1A1*2A have been associated with increased risk of head and neck squamous cell carcinoma [60].

In addition to the role that CYP polymorphisms play in pathological processes and susceptibility to certain diseases, recent genome-wide association studies (GWASs) have demonstrated an association between increased coffee consumption and SNPs rs2472297-T (located between CYP1A1 and CYP1A2) and rs6968865 (next to aryl hydrocarbon receptor) [61]. Huo et al. (2012) determined that certain SNPs are associated with increased susceptibility to schizophrenia [62], while Peñas-Lledó and colleagues found a positive association between the extent of active CYP 2D6 and frequency of suicide attempts, providing evidence that CYP diversity may need to be accounted for in clinical practice [63].

Table 2. Differentially expressed CYPs and their SNP frequencies.

CYP	ID	Mutation	Amino Acid	Global frequency (%)	CYP	ID	Mutation	Amino acid	Global frequency (%)
3A43	rs45450092	435G>T	M145I	1.5	2F1	rs144315434	1172T>C	L391P	5.4
	rs45621431	825G>A	M275I	2.3		rs146029724	1330A>C	M444L	6.5
	rs680055	1018C>G	P340A	13.4	2W1	rs61746347	557G>A	R186H	2.8
	rs78548296	389G>A	R130Q	1.0		rs117826462	547C>G	L183V	1.4
4A11	rs1126743	1374C>G	I458M	42.6	4F11	rs1060463	1271G>A	R424Q	49.5
	rs4926581	553G>T	V185F	28.1		rs148197835	538C>T	R180C	4.2
	rs61736429	1525C>T	L509F	2.1		rs57519667	436C>T	R146C	1.6
	rs62618709	553G>T	V185F	1.1	2C18	rs115091705	431G>A	R144H	1.7
4A22	rs112604161	181G>A	G61R	1.6		rs117111102	370C>T	R124W	1.4
	rs113777592	553G>T	V185F	29.6		rs2281891	1154C>T	T385M	19.3
	rs2056900	388G>A	G130S	29.9		rs41286880	1004G>A	R335Q	2.5
	rs4926600	1525C>T	L509F	12.9		rs79500998	1324C>T	R442C	1.0
	rs61507155	311A>T	Y104F	6.6	7A1	rs8192875	1039G>A	D347N	1.6
	rs61736431	1154C>T	P385L	1.2	4F12	rs16995378	47C>T	T16M	7.6
4B1	rs12094024	986A>C	Y329S	2.2		rs57578760	808G>C	V270L	3.7
	rs2297809	1123C>T	R375C	18.3		rs76142062	88C>A	L30I	3.4
	rs4646487	517C>T	R173W	16.8	11B1	rs11775687	562C>T	P188S	5.7
	rs59694031	1109G>C	C370S	4.0		rs9657020	593C>T	T198M	12.7
4F2	rs2074900	515C>T	Thr172I	25.4	4F3	rs118159249	1420G>A	A474T	1.1
	rs2108622	1297G>A	V433M	20.9	2A7	rs111390860	988C>T	R330W	1.0
	rs3093153	554G>T	G185V	3.7		rs184466431	1301G>T	R434L	1.2
	rs3093200	1555C>A	L519M	8.4		rs3869579	778C>T	R260C	46.7
5A1	rs13306050	1372C>T	R458C	3.3		rs60711313	1259T>C	I420T	3.2
	rs13306052	679GA	V227M	1.4		rs75152309	1106A>T	K369M	6.6
	rs6952940	544C>T	P182S	2.4		rs78754793	244G>C	A82P	2.4
2A6	rs5031017	1436G>T	G479V	1.4	2S1	rs34971233	1397C>T	P466L	1.1
	rs5031016	1412T>C	I471T	5.1	2E1	rs6413419	535G>A	V179I	7.2
	rs28399499	983T>C	I328T	2.3		rs28969387	1370A>T	H457L	6.3
	rs8192709	64C>T	R22C	4.5	2C19	rs17884712	431G>A	R144H	1.4
	rs28399499	383T>C	I128T	2.3	8A1	rs5626	706C>T	R236C	3.7
2C8	rs11572103	805A>T	I269F	16.4	2D6	rs2982054	986G>A	R329H	31.2
	rs1058930	792C>G	I264M	4.1		rs1058172	941G>A	R314H	7.9
	rs11572103	805A>T	I269F	16.4		rs59421388	859G>A	V287M	5.3
2C9	rs28371686	1080C>G	D360E	2.3		rs1065852	100C>T	P34S	25.9
	rs28371685	1003C>T	R335W	2.0	1A1	rs4646422	134GA	G45D	6.7
	rs2256871	752A>G	H251R	4.0		rs17861094	233T>C	I78Thr	8.3

Data was extracted from the 1,000 Genomes Project site (http://www.1000genomes.org/) [23]. A more detailed table can be found in the supplemental material. Possibly CYP-activity damaging SNPs are included in Table S2.

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Diversity of expression in human tissues

Variable expression of functionally distinct CYP isoforms across different tissue types indicates that certain isoforms play specific roles in a tissue-dependent manner. Figure 6 provides an illustrative overview of CYP expression in the human body. Such knowledge may be useful for development of new prodrugs activated by a specific CYP highly expressed in the preferentially targeted tissue, ultimately leading to increased bioavailability at the target site and reduced side effects. On the other hand, variable expression of CYPs in different tissues may adversely affect drug efficacy in some tissues. Such a case could occur if drugs undergo an inactivation through a higher expressed CYP in their target tissues. Regardless, further clinical investigation is required.

Even polymorphic CYP isoforms show a heterogeneous tissue distribution. In particular, CYP 1A2, 2C19 and 2D6 are highly expressed in the pituitary gland. Furthermore, highest expression of CYP 2C9 was detected in the cerebellum, while greatest expression of 2D6 and 2B6 were found in skeletal muscle and kidneys. The influence of mutations in CYPs in particular organs remains to be determined and requires further investigation.

Differential distribution of CYPs may have an influence on specific side effects of drugs. For example, cyclophosphamide (CPA) therapy can lead to development of hyponatremia. CPA is a prodrug converted by CYP 2B6 into the active form [64].

The hyponatremia is the result of increased expression of aquaporins 1 and 7, which is induced by CPA [65]. CYP 2B6 has high expression in kidneys, indicating that a higher level of active CPA is likely to occur in the kidneys and lead to the undesirable side effect.

Conclusions

In summary, the current study identified four major CYPs (1A2, 2D6, 2C9 and 2C19) and 34 polymorphic alleles with a significant impact on the drug metabolism in the Caucasian population. Once genomic testing becomes part of routine analysis, this data enables prediction of complications in drug therapy and development of a personalized treatment regimen, where drug dosages are based on an individual's specific CYP profile [6]. Ultimately, this approach may prevent treatment failures and avoid unnecessary side effects. Another interesting field could be the consideration of CYP polymorphisms in clinical trials. Potentially, it would decrease the failures if information of potential polymorphisms in different ethnic groups was included. Findings from the current study will be included in the SuperCYP database.

With the aim of assessing the effects of CYP polymorphisms on chemotherapy and establishing a cost efficient method to detect relevant CYP polymorphisms, a retrospective study in leukemia cells from pediatric patients is currently under way [66].

Supporting Information

Table S1. Extended table of CYP SNP frequencies in Caucasians and other ethnics. The table includes the content of Table 1 and further information (other ethnics and nucleotide changes) that have been extracted by text mining.
(XLSX)

Table S2. Extended table of differentially expressed CYPs and their SNP frequencies. The extended table lists possibly CYP-activity damaging SNPs. CYP SNPs with frequencies (%) greater than one percent were included. The data was extracted from the 1,000 Genomes Project site (http://www.1000genomes.org/).
(XLSX)

Author Contributions

Conceived and designed the experiments: RP. Performed the experiments: SCP SP MFH AG. Analyzed the data: SCP SP MFH RP MD. Contributed reagents/materials/analysis tools: MD AG. Wrote the manuscript: SP RP SCP MFH.

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Drug Cocktail Optimization in Chemotherapy of Cancer

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Abstract

Background: In general, drug metabolism has to be considered to avoid adverse effects and ineffective therapy. In particular, chemotherapeutic drug cocktails strain drug metabolizing enzymes especially the cytochrome P450 family (CYP). Furthermore, a number of important chemotherapeutic drugs such as cyclophosphamide, ifosfamide, tamoxifen or procarbazine are administered as prodrugs and have to be activated by CYP. Therefore, the genetic variability of these enzymes should be taken into account to design appropriate therapeutic regimens to avoid inadequate drug administration, toxicity and inefficiency.

Objective: The aim of this work was to find drug interactions and to avoid side effects or ineffective therapy in chemotherapy.

Data sources and methods: Information on drug administration in the therapy of leukemia and their drug metabolism was collected from scientific literature and various web resources. We carried out an automated textmining approach. Abstracts of PubMed were filtered for relevant articles using specific keywords. Abstracts were automatically screened for antineoplastic drugs and their synonyms in combination with a set of human CYPs in title or abstract.

Results: We present a comprehensive analysis of over 100 common cancer treatment regimens regarding drug-drug interactions and present alternatives avoiding CYP overload. Typical concomitant medication, e.g. antiemetics or antibiotics is a preferred subject to improvement. A webtool, which allows drug cocktail optimization was developed and is publicly available on http://bioinformatics.charite.de/chemotherapy.

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Introduction

Drug metabolism and drug-drug interactions

Drug metabolism is a complex biochemical network, which consists of many different reactions and pathways in the human organism. Some drugs are excreted unchanged in urine and faeces without metabolic degradation in the liver, but most drugs undergo a multi-step metabolism, which is mainly accomplished by enzymes of the cytochrome P450 family (CYP). CYP catalyze a large amount of enzymatic reactions, such as alcohol oxidations, dehydrogenation and isomerizations. It is a difficult task of medical science and daily clinical practice to establish effective combinations of drugs that do not affect each other's metabolic pathways.

The Human Genome Project revealed 57 different CYP variants [1]. The variant biological activities and specificity among each single CYP are an important issue for researchers as well as physicians. The knowledge of level and catalytic activity of the specific CYP and the effect on drug metabolism could and should lead to personalized drug dosages to optimize the therapeutic effect and minimize harmful side effects. Furthermore, the induction of a CYP by a drug, which is also active in another drug's metabolism, requires increase of the dosage of the first drug to achieve the same therapeutic effect. In case of inhibition, the dosage should be reduced, resulting in diminished side effects. In addition, the drug excretion pathway through kidney has also an important influence on individual drug response. Unfortunately, drugs that are mainly removed by this pathway from the body, will accumulate if an impaired kidney function exists. Therefore, the extrarenal fraction ($\mathbf{Q_0}$) value is able to predict whether a drug is primarily excreted unchanged via kidneys or metabolized and/or removed through another pathway. Thereby is (1- $\mathbf{Q_0}$) the fraction, which is removed unchanged via kidneys. High $\mathbf{Q_0}$ values stand for mainly metabolized drugs and/or kidney independent excretion. In order to prevent adverse side effects and toxic drug levels in diseased kidney patients the $\mathbf{Q_0}$ value should be taken into account to change the drug or adjust the dosage.

Due to multi-drug administration in polychemotherapeutic regimens, adverse side effects are discussed intensely in pharmaceutical research [2]. Three frequently occurring problems should be considered:

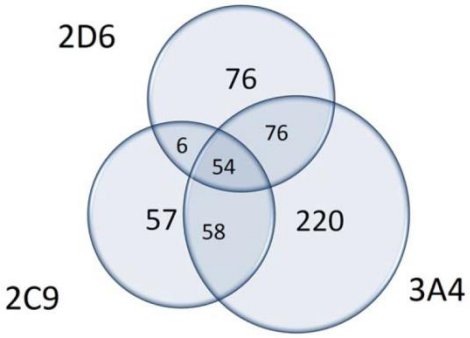

Figure 1. The Venn diagram illustrates the enzyme overload of CYPs 3A4, 2C9, 2D6 in chemotherapy. The numbers within the circles represent the drugs, which are metabolized by the CYPs. Intersection areas show the drugs, which are metabolized by two or three of the CYPs.
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1. Adverse side effects because of limited capacity of metabolizing enzymes,
2. Malfunctioning in-vivo activation of prodrugs due to inhibited or mutated CYPs,
3. Unexpected drug levels because of enzyme induction or inhibition.

CYPs in chemotherapy

In this manuscript, we focus on leukemia while other types of cancer (soft-tissue sarcoma, osteosarcoma, nephroblastoma, neuroblastoma, brain tumors, hodgkin-lymphoma, non-hodgkin lymphoma, low-grade glyoma, and germ cell tumors) are considered at the website.

Most subtypes of leukemia are primarily treated with risk-adapted polychemotherapy protocols, which consist of induction, consolidation, re-induction and maintenance regimens. For risk-adaptation certain prognostic factors are applied, such as leukocyte cell count, age, gender, cytogenetic findings and response to induction therapy [3]. Patients receive up to 13 different antineoplastic drugs. In leukemia, disease progression can be influenced by genetic variants encoding proteases, angiogenic factors, hematopoietic cytokines, bone marrow stroma factors or structural proteins in epithelium. Due to scientific progress individualized medicine is being increasingly developed in the last years and CYP-drug, as well as drug-drug interactions are being considered [4,5]. Individualized medicine also deals with single nucleotide polymorphisms (SNPs) of CYPs to predict patient responses [6,7].

In children with acute lymphoblastic leukemia (ALL) an increased risk of vincristine polyneurotoxicity associated with low CYP 3A5 expression has been reported [8].

Many antineoplastic agents are prodrugs, e.g. cyclophosphamide, ifosfamide, dacarbazine, procarbazine and tamoxifen, requiring in vivo activation by CYPs [9]. An inhibition of CYPs due to multidrug administration could potentially affect negatively the therapeutic efficacy. The clinical relevance of such consider-

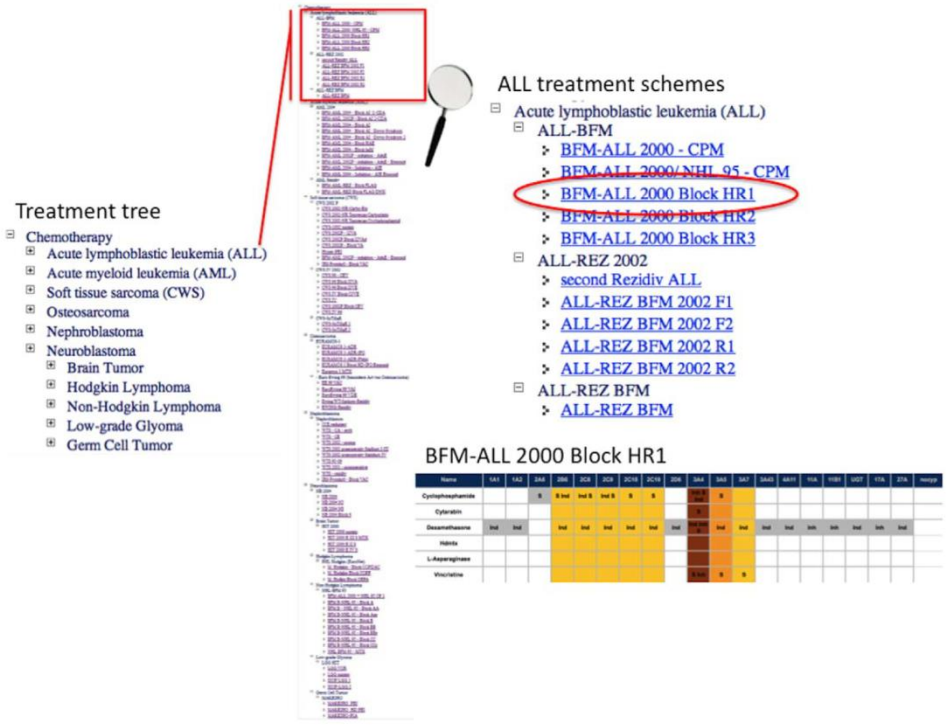

Figure 2. Treatment algorithm: Different antineoplastic treatment regimens in chemotherapy, ordered by diseases. By clicking on one of the diseases, different treatment options open up. After choosing one treatment regimen the metabolism of that drug-cocktail is illustrated.
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Table 1. Treatment regimen before optimization: Drugs for the treatment of ALL at initial diagnosis.

				Involved CYPs			
Drug	**Purpose**	**Q_0**	**EHL**	**Substrate of**	**Inducer of**	**Inhibitor of**	**References**
Cyclophosphamide	Antineoplastic agent	0.75	7	2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 3A4, 3A5	2B6, 2C8, 2C9, 3A4	3A4	[23,24,25,26,27]
Cytarabine	Antineoplastic agent	0.9	2	3A4			[28]
Dexamethasone	Corticosteroid	0.9	3	3A4	1A1, 1A2, 2B6, 2C8, 2C9, 2C18, 2C19, 3A4, 11A, 11B1, 2D6, 3A4, 3A5, 3A7, 3A43, 4A11, UGT, 17A 27A		[17,29,30,31,32,33,34,35,36,37,38,39,40,41]
Vincristine	Antineoplastic agent	0.8	85	3A4, 3A5, 3A7		3A4	[17,42,43,44]
HdMTX	Antineoplastic agent						
L-Asparaginase	Antineoplastic agent						

The second, third and fourth columns list the purpose of these drugs, their extrarenal fraction (Q_0) and elimination half-life (EHL), while the next three columns show involved CYPs ordered by substrate, inducer and inhibitor. References are given in the last column.
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ations was shown in several clinical trials, where CYPs and SNPs play a role in potentially preventing treatment related deaths [9,10,11,12,13]. A retrospective study showed a 3-fold higher risk of death in patients with a polymorphism of CYP3A4 who were receiving cyclophosphamide-based adjuvant chemotherapy [14].

These findings suggest that individual SNPs in CYPs and drug-drug interactions in polychemotherapy are important issues and treatment regimens should be reevaluated regarding such interactions.

Materials and Methods

Treatment regimens

Information on drug administration of chemotherapeuticals in oncology and their drug metabolism was collected from scientific literature and various web resources. About 100 common treatment regimens were extracted from the blue book [15].

CYP-drug interactions

The drugs from the treatment regimens were subdivided into two groups regarding their purpose:

a) Antineoplastic agents

b) Supportive treatment, e.g. antiemetics, antimycotics, antibiotics

Information on CYP metabolism was also extracted from Nelsons Homepage [16], Flockharts Interaction Table [17], University of Maryland's Drug Checker, PubChem [18], PDB [19]. Some information was gathered from FDA-files.

Textmining

The flood of information on drugs in the world wide web (WWW) is overwhelming [20]. The World Wide Web Cosortium aims at converting the existing web into a Semantic Web or "web of data" [21]. Accordingly, we carried out a new textmining approach using Semantic Web Standards. For the development of the CYP-specialized textmining pipeline we used the literature and information retrieval packages Lucene and LingPipe. Therefore, the complete Medline/PubMed data was downloaded from the NCBI FTP site in xml-format and indexed. The indexed data is dynamically queried by a search engine written in Java resulting in a sql-file containing the textmining hits. The search engine comprises several lists of synonyms for identifying entities like chemical compounds, biological targets, genes, cell-types, polymorphisms as well as interaction related entities. Abstracts were automatically screened for antineoplastic drugs and their synonyms in combination with a set of human CYPs in title or abstract. Furthermore, the relation between drug and CYP was classified according to interaction terms like "inhibit", "induce", "metabolize" etc. The query was: (DrugSynonym[ti] AND CypSynonym[ti]) OR (DrugSynonym[abstract] AND InteractionTerm AND CypSynonym[abstract]). There was a need for restricting positional distance between occurrences of the terms, e.g. if terms are found far from each other in a paper. Those 2,060 records found were scored rule-based to identify relations between entities. The rules employ order, redundancy and distance between entities, topic segmentation and sentence breaking for boundaries. Duplicates were removed and a team of scientists manually processed 723 papers found in PubMed. Each drug was attributed to those CYPs that are involved in drug metabolism as substrate, inhibitor or inducer.

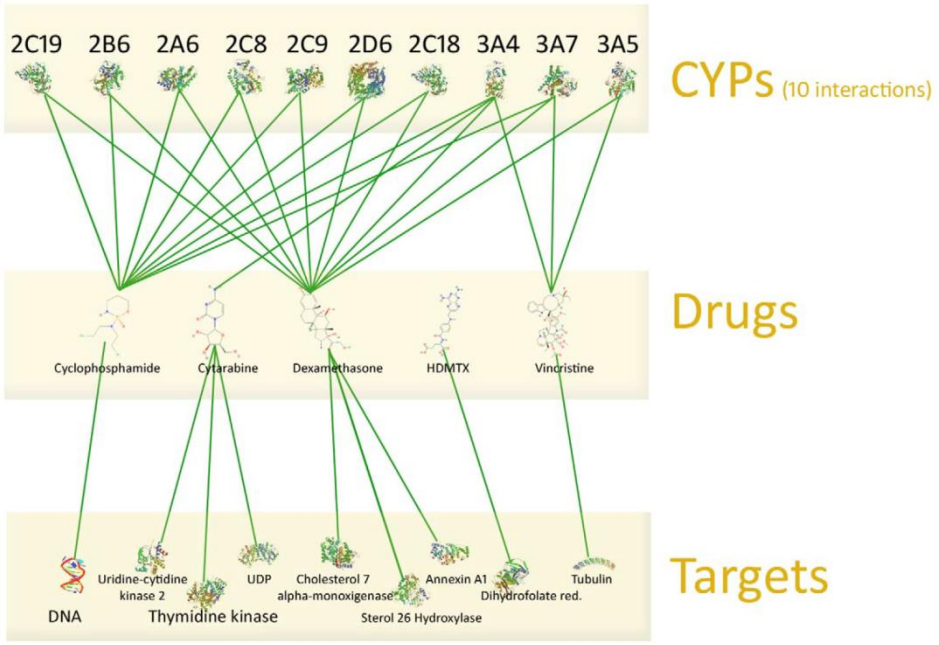

Figure 3. CYP interactions and targets of treatment regimen before optimization. The drugs of the medication are listed centrally in the Figure. Several green lines heading upwards illustrate ten CYPs, which are involved in the metabolism. The green lines heading downwards show the targets, which are metabolized by these drugs.
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ATC Classification System

Many problems, such as enzyme overload, enzyme induction or inhibition occur in combination therapy of leukemia. Some of these drug-drug interactions can be avoided by choosing an alternative drug. Based on the WHO classification system, that classifies drugs into different groups according to *A*natomic site of action, *T*herapeutical effect and *C*hemical structure (ATC), alternative drugs could be administered. Additionally, the suggestions of alternative drugs were manually curated by oncologists and checked for sanity.

Database

To overcome these problems, we generated a web-interface for clinicians to check drug-drug interactions. The database provides information on drug metabolism including PubMed references. The database is designed as a relational database on a MySQL server. For chemical functionality, the MyChem package is included, which aims to provide a complete set of functions for handling chemical data within MySQL. The website is built with PHP and javascript, web access is enabled via Apache Webserver 2.2.

Results and Discussion

Those 2,060 records were found through the automated textmining approach. Another 50 records were manually identified. 864 duplicates were automatically removed and another 92 records were excluded. A team of scientists manually processed 723 papers found in PubMed. There are a lot of undesired drug-drug interactions via CYPs. In particular, the number and effect of anti-neoplastic drugs often cause severe problems, possibly ending up with death. The extensive search revealed three CYPs, which are mainly involved in the metabolism of antineoplastic agents.

Figure 1 shows these CYPs, namely CYP 3A4, 2D6 and 2C9, which are involved in the metabolism of most of the drugs. Interestingly, CYPs 2D6 and 2C9 are highly polymorphic, which makes it even more important to disencumber the CYPs from some drugs and in second step, trying to use different metabolic pathways.

We have analyzed the antineoplastic drugs from over 100 treatment regimens regarding their drug metabolism. The results are summarized in Table S1 of Supporting Information.

To optimize therapeutic regimens, the effect of supportive drugs like antibiotics, antimycotics, antiemetics etc. in the metabolic process have to be taken into account, which are shown in Table S2 of Supporting Information.

These analyses suggest several drug-drug interactions, but also show some alternatives to avoid enzyme overload or induction. Additionally, the analysis of the ATC codes for drug classification and the addressed targets provide hints for possible alternative medication. Going through the list of chemotherapeutic drugs and supportive medication, we have compiled a comprehensive list of combination therapies, which are optimized regarding their metabolism. This list is structured according to an algorithm starting from the different cancer types, different therapy cycles, relapse etc. (Figure 2).

Furthermore, the $\mathbf{Q_0}$ and elimination half-life (EHL) values are displayed to compare the pharmacological properties of drugs and their alternatives. On the one hand, longer EHL potentially means CYP overload and should be avoided, on the other hand the effective presence of the drugs has to be longer than the cell cycle of the cancer cells (re-dosing may be required for shorter EHLs).

Table 2. Treatment regimen after optimization: Possible alternatives in the treatment of ALL.

				Involved CYPs			
Drug	**Purpose**	**Q_0**	**EHL**	**Substrate of**	**Inducer of**	**Inhibitor of**	**References**
Gemcitabine	Antineoplastic agent	0.9	1.2				
Prednisolone	Corticosteroid	0.7	3	3A4, 3A5	3A4, 3A5	2A6	[45,46,47,48]
Trofosfamide	Antineoplastic agent	0.9	1	2B6, 3A4			[49]
Vindesine	Antineoplastic agent	0.87	24	3A4			[50]
HdMTX	Antineoplastic agent						
L-Asparagine	Antineoplastic agent						

The second, third and fourth columns list the purpose of these drugs, their extrarenal fraction (Q_0) and elimination half-life (EHL) in hours, while the next three columns show involved CYPs ordered by substrate, inducer and inhibitor. References are given in the last column.
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The consideration of individual pharmacokinetic parameters like Km and Vmax for drugs and CYPs [22] would be desirable but requires refined models for each particular drug-drug interaction (reversible, competitive, non-competitive, uncompetitive, irreversible etc.), which remains a future goal. Beside the role in patients with nephropathies, the Q_0 could also help to estimate the extent of CYP-drug interactions. Drugs with low Q_0 values (<0.3) are excreted unchanged to a large extent and occupying the CYP system lesser. In conclusion, their impact on interactions is lower than for drugs with higher Q_0 values. Hence, consideration of Q_0 values in finding alternative drugs is useful to reduce the interaction potential, if the function of kidneys is sufficient. However, limitations are a small number of eligible drugs with low Q_0 values, and that high values do not necessarily mean more CYP reactions. But it provides a useful support to select the alternative drugs.

To exemplify here, we chose one typical treatment protocol for the treatment of ALL, which consists of the antineoplastic drugs cyclophosphamide, cytarabine, HDMTX, L-asparaginase and vincristine, as well as the corticosteroids prednisone/dexamethasone.

CYPs involved in the metabolism of the mentioned drugs are listed in Table 1, ordered by substrate, inducer and inhibitor. It is clearly visible, that many CYPs are involved in the metabolism processes, ending up in eleven interactions. These are illustrated with targets in Figure 3.

Based on the ATC codes, we extracted an alternative treatment regimen to avoid these interactions. The results are illustrated in Table 2 and Figure 4. Figure 4 shows that there is only one

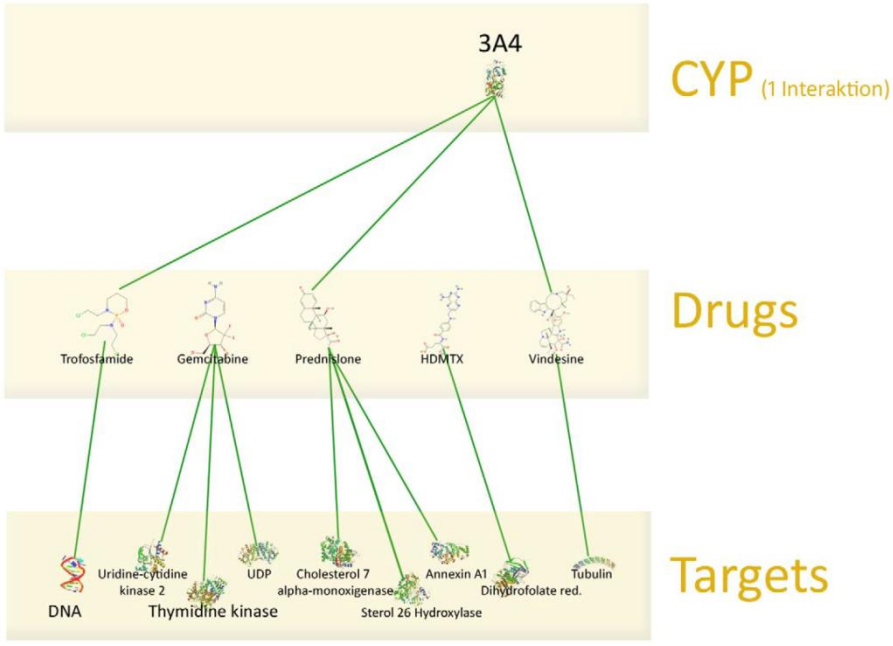

Figure 4. CYP interactions and targets of treatment regimen after optimization. By choosing drugs from the same ATC group with different metabolism pathways, only one CYP interaction remains.
doi:10.1371/journal.pone.0051020.g004

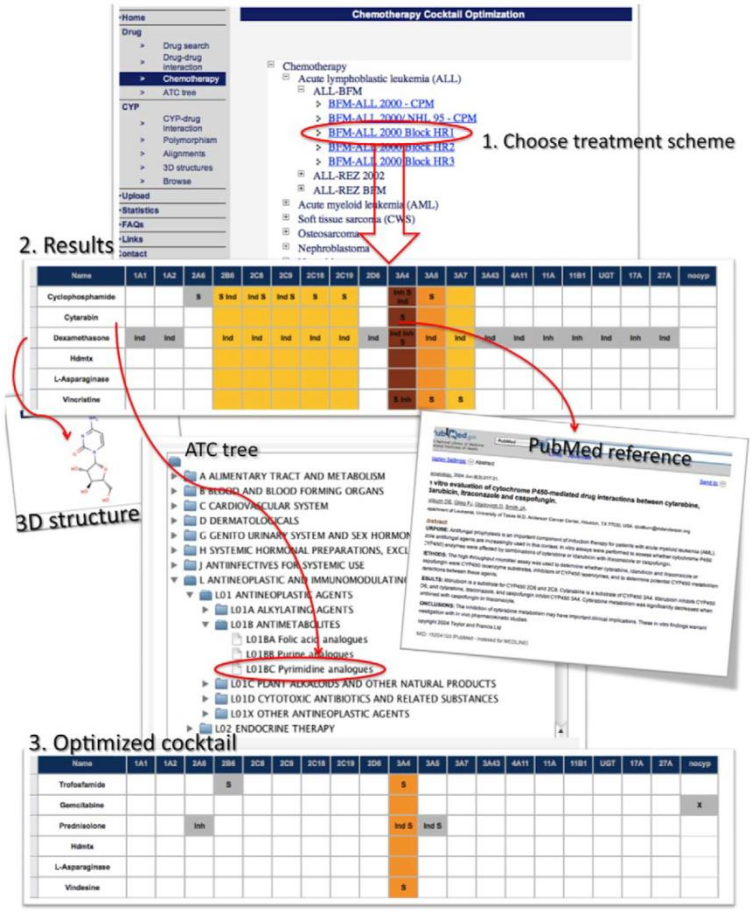

Figure 5. Optimization tool. Clicking on "Chemotherapy" in the navigation directs to the treatment tree, enabling to browse through different treatment regimens ordered by diseases. Once a treatment regimen is chosen, the drug-cocktail is shown on the "Results" page. The enzyme overload is visualized in different colors. PubMed references are indicated, as well as 3D structures of the drugs and the ATC tree defining the purpose of the drugs. Based on the ATC group, several alternatives for each drug are given, providing optimization of the cocktail with less drug-drug-interactions.
doi:10.1371/journal.pone.0051020.g005

interaction left, while all other interactions could be omitted using different metabolic pathways of other drugs.

Database

We created a web-tool for clinicians to analyze diverse drug-drug interactions of over 100 antineoplastic treatment regimens. Figure 5 shows the main features of the website. To visualize treatment regimens, just click on "Chemotherapy" in the navigation. If your specific drug-cocktail is not in the list, click on "Drug-drug interaction" and type in your medication manually. Once a treatment regimen is chosen or manually typed a drug-cocktail, the database provides a variety of information.

To view drug structures or ATC groups, just click on the drug. CYPs involved in the same metabolic pathway are presented in different columns. "S" means substrate, "E" inducer and "I" inhibitor. Clicking on these abbreviations leads to the PubMed references. Colored columns illustrate the multi-use of specific CYP pathways. Based on ATC-codes, drug alternatives using different metabolic pathways for each drug are presented below, which enables the user to optimize the cocktail regarding its metabolism.

This comprehensive resource is freely available at: http://bioinformatics.charite.de/chemotherapy and is also applicable on smartphones and tablet-PCs.

Supporting Information

Table S1 Antineoplastic drugs in polychemotherapy regimens. Involved CYPs are ordered by substrate "S", inducer "E" and inhibitor "I".
(DOCX)

Table S2 Supportive treatment used in chemotherapy. Involved CYPs are ordered by substrate "S", inducer "E" and inhibitor "I".
(DOCX)

Author Contributions

Conceived and designed the experiments: RP SP. Performed the experiments: MFH SP SCP. Analyzed the data: MD WWR NG. Contributed reagents/materials/analysis tools: KS. Wrote the paper: SP RP KS.

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4 Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

5 Publikationsliste

Michael F. Hoffmann*, Sarah C. Preissner*, Janette Nickel, Mathias Dunkel, Robert Preissner, Saskia Preissner. *The Transformer database: biotransformation of xenobiotics.* Nucleic Acids Research 2014 Jan; 42(Database issue):D1113-7. [Epub 2013 Dec 10]

* geteilte Erstautoren Impact Factor: 8,278

Sarah C. Preissner*, **Michael F. Hoffmann***, Robert Preissner, Mathias Dunkel, Andreas Gewiess, Saskia Preissner. *Polymorphic Cytochrome P450 Enzymes (CYPs) and Their Role in Personalized Therapy.* PLoS One 2013 Dec 10; 8(12):e82562.

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Saskia Preissner, Mathias Dunkel, **Michael F. Hoffmann**, Sarah C. Preissner, Nikolai Genov, Wen Wei Rong, Robert Preissner, Karlheinz Seeger. *Drug Cocktail Optimization in Chemotherapy of Cancer.* PLoS One 2012; 7(12):e51020. [Epub 2012 Dec 7]

Impact Factor: 3,730

Joachim von Eichborn, Mathias Dunkel, Björn O. Gohlke, Sarah C. Preissner, **Michael F. Hoffmann**, Jakob M. J. Bauer, J. D. Armstrong, Martin H. Schaefer, Miguel A. Andrade-Navarro, Nicolas Le Novere, Michael D. R. Croning, Seth G. N. Grant, Pim van Nierop, August B. Smit, Robert Preissner. *SynSysNet: integration of experimental data on synaptic protein-protein interactions with drug-target relations.* Nucleic Acids Research 2013 Jan; 41(Database issue):D834-40. [Epub 2012 Nov 11]

Impact Factor: 8,278

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