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H A B I L I T A T I O N S S C H R I F T

Systemmedizin des Zahnschmerzes -

Berücksichtigung von Rezeptoren, individueller Komedikation und relevanter genetischer Polymorphismen

zur Erlangung der Lehrbefähigung für das Fach
Zahn-, Mund- und Kieferheilkunde

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Inhaltsverzeichnis

1.Einleitung

1.1 Systembiologie und Systemmedizin

Die *Systembiologie* ist eine junge Wissenschaft, die es sich zur Aufgabe gemacht hat biologische Organismen in ihrer Gesamtheit zu erklären. Es gibt, je nach Fachgebiet, leicht abweichende Definitionen, aber die Begriffe „integrativ“ und „ganzheitlich“ werden zur Beschreibung der Ansätze einheitlich verwendet. Dabei bezieht sich „ganzheitlich“ sowohl auf die Betrachtung vieler paralleler Prozesse als auch auf die Betrachtung unterschiedlicher Organisationsniveaus (vom Atom über biochemische Reaktionen und Organellen bis zum gesamten Organismus). Ein wichtiger Teilaspekt ist die Betrachtung der Zeitabhängigkeit von Prozessen. Der „Multi-Skalen-Gedanke“ wird also von der räumlichen Komponente auf die Zeitachse ausgedehnt, wodurch die Erklärung von Langzeiteffekten erst möglich wird.

Das Beschreibungsniveau der Systembiologie hat inzwischen ein Entwicklungsstadium erreicht, in dem die medizinische Anwendung realistisch wird, weshalb u.a. die Europäische Union verschiedene Förderprogramme – z.B. *virtual physiological human* – aufgelegt hat. Die *Systemmedizin* nutzt die Ansätze und Methoden der Systembiologie, um komplexe kausale Zusammenhänge (z.B. genetische Regulationsnetzwerke) zu erfassen, die für pathologische Zustände verantwortlich sind. In diesem Zusammenhang spielen sogenannte „*omics*-Technologien“ (Metabolomics, Proteomics usw.) eine wichtige Rolle, denn ein (großes) biochemisches Reaktionsnetzwerk kann man nur verstehen, wenn man möglichst alle Metabolite zu verschiedenen Zeitpunkten misst und mit den zugehörigen Enzymkonzentrationen korreliert. Für die Systemmedizin ist also eine enge Verzahnung (Zyklen) von Labor und Klinik (Therapie) besonders wichtig.

1.2 Schmerz – ein ganzheitlich-systemisches Phänomen

Der Schmerz muss aus verschiedenen Gründen als ganzheitlich-systemisches Phänomen behandelt werden. Einerseits ist der Schmerz ein essenzielles Element, um verschiedenen Gefahren adäquat zu begegnen, andererseits kann der Schmerz zu einer massiven Verschlechterung der Lebensqualität führen. Bei jedem Schmerzereignis müssen somatische, psychologische, soziale Faktoren berücksichtigt werden. Die Lehrbücher der medizinischen Grundlagenfächer belegen die komplette Durchdringung des menschlichen Körpers mit Sensoren und Leitungssystemen des Schmerzes. Dabei spielen sensorische, nervale, senso-motorische, humorale, elektromagnetische und vegetative Informationskanäle eine Rolle. So kann beispielsweise ein Schmerzzustand außerhalb des orofazialen Systems entstehen, indem eine Fehlbisslage den Muskeltonus der Kaumuskulatur verändert, wodurch wiederum – über neuronale und musko-skelettale Vernetzungen – Veränderungen in der Kopf- oder Körperhaltung auftreten, was ebenfalls umgekehrt möglich ist.

1.3 Chronifizierung

Bei der Schmerzchronifizierung finden neuroplastische Veränderungen statt, sodass die vollständige Heilung mitunter nicht möglich ist. Dieses Problem wird in der Zahnmedizin häufig unterschätzt. Durch einen länger andauernden exzitatorischen Reiz, werden zum Beispiel vermehrt spannungsabhängige Natrium- und Kalziumkanäle eingebaut, die die Erregbarkeit der Synapse verstärken. Dadurch kann es zur Degeneration nozizeptiver oder hemmender Systeme kommen. Im Bereich der Zahnmedizin ist eine chronische Hypersensibilisierung jedoch der häufigste pathogene Mechanismus des chronifizierten Schmerzes. Bei der Präparation vitaler Zähne für Kronen oder Brücken wird häufig auf eine Lokalanästhesie verzichtet, da es sich um einen relativ kleinen Eingriff handelt. Hier entstehen jedoch bei hohem Anpressdruck, schnellen Drehzahlen von bis zu 200.000 Umdrehungen pro Minute und nicht ausreichender Wasserkühlung

mechanische und thermische Reize, die zu einer Irritation der Pulpa führen. Patienten leiden oft noch Monate nach dem Einsetzen einer Krone an Schmerzen durch Berührung, Druck oder Temperatur, die nicht selten eine Wurzelkanalbehandlung zur Folge haben. Auch bei anderen zahnärztlichen Eingriffen, wie dem Exkavieren von Karies, dem Anfertigen von Füllungen, exzessiven Trocknungsmaßnahmen usw. werden Hypersensibilisierungen beobachtet, wobei dies häufig von Zahnärzten bagatellisiert wird. Aufgrund der Gefahr der irreversiblen Chronifizierung des Schmerzes sollte daher nicht auf eine suffiziente Lokalanästhesie verzichtet werden.

1.4 Spezifika des Zahnschmerzes

Neben den beschriebenen Reizen ist die am häufigsten auftretende Ursache für Zahnschmerzen die irreversible Pulpitis, der meist ein kariöser Prozess voran geht. Der kariogene Säureangriff wird durch Bakterien in der Plaque initiiert, die Kohlenhydrate durch Glykolyse zu Laktat metabolisieren, was eine Absenkung des pH-Wertes zur Folge hat. Ein pH-Wert unter 5,5 wird für den Zahnschmelz als kritisch bezeichnet, da hier ein Herauslösen von Mineralien möglich ist (1). Häufig bemerken Patienten in frühen Stadien leichte Schmerzen bei besonders süßen, heißen oder kalten Nahrungsmitteln. Schreitet dieser Demineralisationsprozess voran, kommt es zu einer bakteriellen Invasion der Pulpa, die eine Pulpitis zur Folge hat. Ist der Schmerz reizüberdauernd und tritt auch nachts auf, spricht man von einer irreversiblen Pulpitis (2). Patienten suchen dann häufig nachts den zahnärztlichen Notdienst auf, da Schmerzmedikamente wie Ibuprofen und Paracetamol keine Wirkung mehr zeigen und lediglich die kühlende Wirkung des schluckweisen Trinkens von kaltem Wasser eine kurzfristige Linderung der Schmerzen bringt. Bereits die Lokalanästhesie bringt den Patienten Linderung, wobei die kausale Therapie in der Regel eine Wurzelkanalbehandlung ist, bei der der Zahn eröffnet wird um nekrotisches und infiziertes Gewebe zu entfernen. Neben den beschriebenen klassischen Zahnschmerzen gibt es eine Vielzahl von

anderen Schmerzformen, die von den Zähnen, vom Zahnhalteapparat, der Kieferhöhle, dem Kiefergelenk oder der Kaumuskulatur ausgehen können. Eine umfangreiche Diagnostik ist daher grundsätzlich vor jeder Therapie unerlässlich.

1.5 Schmerzdiagnostik

Es gibt unterschiedliche Formen von Schmerz, wobei man im Wesentlichen zwischen nozizeptivem, neuropathischem, psychogenem und idiopathischem Schmerz unterscheidet. Der nozizeptive Schmerz entsteht durch die Aktivierung von Nozizeptoren, die auf einen Reiz, wie zum Beispiel eine Gewebedestruktion reagieren. Hier werden somatischer (von Gelenken, Knochen, Muskeln) und viszeraler (von inneren Organen) Schmerz unterschieden. Die bekannteste Form des neuropathischen Schmerzes ist die diabetische Neuropathie. Allen Formen des neuropathischen Schmerzes liegt eine Irritation oder Schädigung des somatosensorischen Nervensystems zugrunde. Durch Depressionen oder Angstzustände kann es zu so genannten psychogenen Schmerzen kommen, die meist keinen somatischen Ursprung haben, sodass die Behandlung dieser Schmerzpatienten eine besonders große Herausforderung darstellt. Auch die Therapie von idiopathischen Schmerzen ist schwierig und muss meist multifaktoriell sein, da betroffene Patienten häufig Schmerzen aufgrund einer Schmerzvorgeschichte (Kiefergelenksbeschwerden oder Fibromyalgie) entwickeln.
Bei der Diagnostik ist es wichtig zu beachten, dass sämtliche Schmerzformen im orofazialen Bereich auftreten können, wobei die meisten und häufigsten Formen des Zahnschmerzes dem nozizeptiven Schmerz zugeordnet werden. In der Regel wird man zunächst eine detaillierte allgemeine und spezielle (Schmerz-)Anamnese erheben, um erste Hinweise für die Ursache der Schmerzen zu erhalten. Viele Patienten nehmen regelmäßig Medikamente ein und haben sich vor dem Besuch beim Zahnarzt eigenständig mit Schmerzmedikamenten prämediziert. Dies ist wichtig zu beachten, da dadurch zum einen die Schmerzwahrnehmung gestört sein kann (3) und zum anderen bei der Einnahme von Marcumar oder Thrombozyten-

aggregationshemmern wie Acetylsalicylsäure oder Clopidogrel von invasiven Eingriffen wie Extraktionen oder Abzessinzisionen bis zur Rücksprache mit dem Hausarzt abgesehen werden sollte (4). Nach Erhebung der Anamnese folgt eine extraorale Untersuchung des Patienten, die das Vorliegen von Schwellungen, vergrößerten Lymphknoten, Fieber u.ä. abklärt. Im Anschluss folgt eine intraorale Untersuchung, bei der Vitalität, Perkussions- und Aufbissempfindlichkeit, Sondierungstiefen und Entzündungsgrad des Zahnhalteapparates überprüft werden. Nach diesen Untersuchungen kann der Zahnarzt in der Regel eine Verdachtsdiagnose aufstellen, die ggf. durch ein Röntgenbild bestätigt werden sollte (z.B. bei apikalen Parodontitiden).

1.6 Schmerztherapie

Die *International Association for the Study of Pain* hat in der Deklaration von Montréal den Zugang zu einer adäquaten Schmerzbehandlung als fundamentales Menschenrecht definiert und in diesem Schriftstück auch die dringende Notwendigkeit zur weiteren Forschung an Schmerz bzw. der Schmerztherapie angemahnt (5). Besonders großer Bedarf besteht insbesondere bei der Behandlung von Patienten in präfinalen Konditionen (Tumorschmerzen) oder Patienten mit langjährigen chronischen Schmerzen.

Die Deutsche Gesellschaft für Zahn-, Mund- und Kieferheilkunde empfiehlt in einer wissenschaftlichen Stellungnahme, sich bei der Behandlung von akuten Schmerzpatienten mit Dolor post extractionem, Dentitio difficilis, Parodontitiden und Pulpitiden im zahnärztlichen Notdienst auf die adäquate Schmerzbeseitigung zu konzentrieren (6).

Je nach Ursache gibt es verschiedene Ansätze in der Schmerztherapie, wobei grundsätzlich verhindert werden sollte, dass Schmerzen chronifizieren, sodass eine suffiziente Lokalanästhesie zu Beginn der Behandlung meist unerlässlich ist (7). Lokalanästhetika blockieren Na^+-Kanäle und setzen so die Erregbarkeit sensibler

und motorischer Nervenendigungen herab, wodurch in der Regel die Schmerzempfindung aufgehoben wird (8,9).

Bei apikalen Parodontitiden ist die Therapie der Wahl eine Wurzelkanalbehandlung (2), bei der das infizierte pulpale Gewebe aus dem Zahn entfernt wird, die Wurzelkanäle gereinigt und desinfiziert werden und lokal ein Medikament appliziert wird. Bei akuten Schmerzen bietet es sich an, ein Kortison-Antibiotika-Kombinationspräparat anzuwenden, da es dann zu einer raschen Verbesserung der Symptome kommt (10). Den Patienten wird meist empfohlen, zusätzlich weiterhin ein nicht-steroidales Antirheumatikum einzunehmen, um eine weitere Schmerzbahnung zu unterbinden (11). Bei Schwellungen mit Tendenz zur Ausbreitung (z.B. Logenabszess) und Fieber ist es ratsam, Patienten zusätzlich antibiotisch abzuschirmen, wobei in der Zahnmedizin Amoxicillin am weitesten verbreitet ist (12) und bei bekannten Unverträglichkeiten auf Clindamycin oder Metronidazol ausgewichen werden kann (13).

Bei Schmerzen, die durch eine Entzündung des marginalen Parodonts verursacht wurden, wird in der Regel versucht, eine lokale Reinigung und Desinfektion der betroffenen Wurzeloberflächen zu erzielen, um dann ein Kortison-Antibiotika-Kombinationspräparat einzubringen. Auch hier muss bei schweren Formen der Parodontitis mitunter systemisch antibiotisch behandelt werden, wobei die Kenntnis der individuellen Komedikation von großer Bedeutung ist (14).

Patienten, die an rheumatoider Arthritis leiden, nehmen häufig nicht-steroidale Antirheumatika (NSAIDs) und/oder Kortikosteroide, krankheitsmodifizierende Antirheumatika (DMARDs) und Biologika ein (15). Häufig beobachtet man bei diesen Patienten auch eine Parodontitis (16). Es konnte herausgefunden werden, dass der *Porphyromonas gingivalis* in der Lage ist, in Endothelzellen und Chondrozyten einzudringen (17,18). Bei der aggressiven Parodontitis kommt es durch die Anwesenheit von bestimmten, besonders pathogenen Keimen zu einer sehr schnellen Knochendestruktion, sodass eine gezielte systemische Antibiotikatherapie sinnvoll ist (14). Bei der Betrachtung des Metabolismus der Medikamente gegen rheumatoide Arthritits und den der Antibiotika gegen die aggressive Parodontitis,

fällt auf, dass daran wenige Enzyme beteiligt sind und es daher schnell zu gegenseitigen Wechselwirkungen kommen kann, die sich negativ auf die Therapie beider Erkrankungen auswirken kann (19). Gleichzeitig kann sich eine Reduktion der parodontalen Infektion günstig auf den Verlauf der rheumatoiden Arthritis auswirken (20).

1.6.1 Medikamentöse Allopathie

Die Behandlung von Schmerzen erfolgt in der Regel stufenweise. Grundsätzlich wird zwischen nicht-opioiden und opioiden Analgetika unterschieden. Innerhalb der nicht-opioden Analgetika wird zusätzlich zwischen sauren antiphlogistischen antipyretischen Analgetika (nicht-steroidalen Antirheumatika), nicht-sauren antipyretische Analgetika und Analgetika ohne antiphlogistische oder antipyretische Wirkung unterschieden.

Da häufig ein Entzündungsprozess zugrunde liegt, sind die nicht-steroidalen Antirheumatika in der Zahnmedizin zunächst Mittel der Wahl (21), wobei von Acetylsalicylsäure (ASS) aufgrund der Thrombozytenaggregationshemmung abgeraten wird. Daher werden vor allem Ibuprofen, Dexketoprofen und Diclofenac empfohlen (22). Bei den nicht-sauren antipyretischen Analgetika kommt in erster Linie Paracetamol zum Einsatz, seltener Metamizol. Nicht-opiode Analgetika ohne antipyretischen oder antiphlogistischen Effekt kommen in der Zahnmedizin nur in seltenen Fällen, wie bei sehr schmerzhaften Muskelverspannungen zum Einsatz. Hier erfolgt in der Regel eine Rücksprache mit dem behandelnden Arzt. Zusätzlich zu den Schmerzmedikamenten, kann eine Komedikation mit Kortikosteroiden, Antidepressiva oder Antiepileptika notwendig sein, die allerdings ebenfalls grundsätzlich hausärztlich abgeklärt werden muss.

1.6.2 Therapeutische Lokalanästhesie

Bei starken Schmerzen kann es mitunter hilfreich sein, die Lokalanästhesie auch therapeutisch einzusetzen. Dies gilt vor allem für Schmerzen, die nicht oder nur sekundär dentogenen Ursprungs sind, wie bei kranio-mandibulären Dysfunktionen (23). Hierfür eignen sich länger wirksame Lokalanästhetika wie Bupivacain und Ropivacain, deren Wirkung sehr schnell einsetzt und Patienten mit Muskel- oder Gelenkschmerzen sofort hilft und damit einer Chronifizierung entgegen wirken kann (24).

1.6.3 Weitere Therapieformen

Nicht immer kann durch medikamentöse oder rein zahnärztliche Therapie eine Schmerzfreiheit erreicht werden. Die Kenntnis der weiteren Behandlungsoptionen ist daher unerlässlich. Bei einigen Patienten mit kranio-mandibulären Dysfunktionen, wie myogenen oder arthrogenen Schmerzen, rezidivierenden Kierfergelenkssubluxationen oder anterioren Diskusverlagerungen bietet es sich an, durch manuelle Therapie oder Physiotherapie Verspannungen oder Fehlbelastungen zu lösen (25). Auch Botulinumtoxin kann bei myofazialen Schmerzen oder fokalen Muskelverspannungen effektiv intraoral eingesetzt werden (26). Auch Akupunktur, Low-Level-Lasertherapie oder homöopathische Ansätze können im Einzelfall erwogen werden.

1.7 Individualisierte Schmerztherapie

Aufgrund der komplexen Zusammenhänge zwischen Schmerzen und der Vielzahl äußerer bzw. innerer Einflüsse ist es erstrebenswert, für jeden Patienten eine individuelle Schmerztherapie zu erarbeiten, die sowohl die Bedürfnisse und Wünsche des Patienten, als auch seine Komedikation und etwaige genetische Polymorphismen berücksichtigt. In einigen wenigen Bereichen der Medizin wird dies bereits teilweise praktiziert. Da ein Großteil anti-psychotischer Medikamente

über die hoch polymorphen CYPs 2D6 und 2C19 metabolisiert wird, ist die Genotypisierung in der Psychiatrie schon weiter verbreitet, um die Dosierung entsprechend der Aktivität der CYPs anzupassen, wodurch nicht nur unerwünschte Nebenwirkungen oder Unwirksamkeit verhindert werden, sondern auch die mitunter langen stationären Einstellzeiten deutlich reduziert werden können (27,28). In der Zahnmedizin hingegen werden Schmerzen häufig bagatellisiert, wodurch es nicht selten zur Chronifizierung kommt. Auf Basis von allgemein gültigen lange bewährten Therapieformen behandelt, bleibt eine Besserung aus, werden betroffene Zähne extrahiert und bleibt die Besserung weiterhin aus, wird Patienten nahegelegt, sich in psychotherapeutische Behandlung zu begeben. Hier besteht daher ein besonders großer Bedarf an Aufklärung und weiterer Forschung zur Prophylaxe und Behandlung von Schmerzen (29).

2. Eigene Arbeiten

2.1 Ionenkanäle als aussichtsreiche Targets der Schmerzhemmung

Für die Schmerztherapie gibt es eine Reihe etablierter Zielmoleküle, häufig Ionenkanäle. Ionenkanäle sind Proteine, die spannungs- oder ligandenabhängig einen Ionenstrom zulassen oder unterbinden. Die Familie der humanen TRP-Kanäle (engl. transient receptor potential) besteht aus 28 zellulären Ionenkanälen, die alle nonselektiv permeabel für Kationen sind (30). Verbindungen wie Capsaicin aus der Chili-Schote, die als „scharf“ empfunden werden, stimulieren den Vanilloid-Rezeptor TRPV1. Die Abbildung 1 zeigt eine mögliche Docking-Position von Capsaicin an TRPV1, wie sie im Rahmen einer Studie erarbeitet wurde (31).

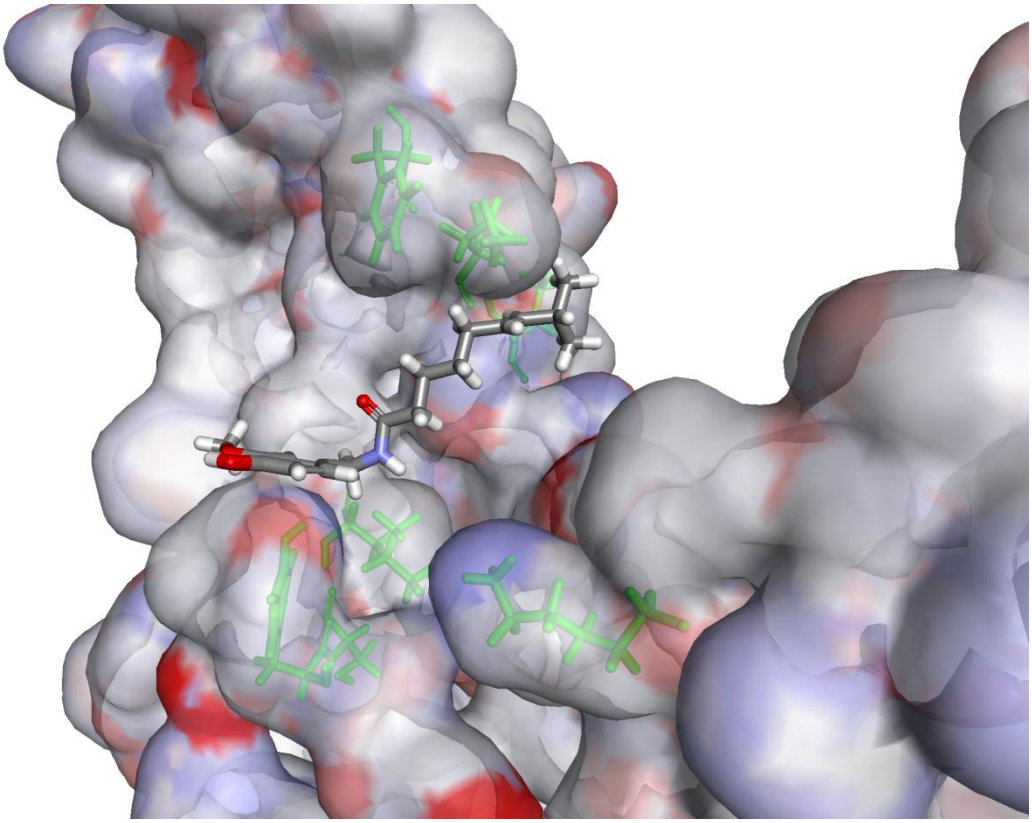

Abbildung 1: **Capsaicin an TRPV1.** Das entwickelte Modell des TRPV1-Rezeptors wurde genutzt, um eine energetisch günstige Bindungsstelle für das Capsaicin zu finden.

Dieser Rezeptor spielt auch eine entscheidende Rolle bei der Schmerzvermittlung und ist daher ein interessantes Target für die Entwicklung von

Schmerzmedikamenten (32). Zunächst wurden hoch affine Verbindungen untersucht, während man inzwischen eher nach Verbindungen sucht, die eine allosterische Modulation bewirken oder ein breites Spektrum von TRP-Kanälen hemmen (33,34). Im Rahmen des Projekts wurden alle experimentell bestätigten Verbindungen an die verschiedenen Rezeptoren gedockt, um eine Vorstellung von den Bindungsmechanismen entwickeln und gezielter nach neuen Verbindungen suchen zu können. Außerdem ergeben sich aus den Bindungsbreichen Hinweise auf relevante Einzelnukleotid-Polymorphismen (SNPs).

Bis zur Entdeckung des Menthol-Rezeptors (TRPM8) war der Mechanismus der Kälte-induzierten Schmerzhemmung unklar. Der Rezeptor wird durch Kälte bzw. kühlende Agenzien, wie Menthol oder Icilin aktiviert, wodurch Natrium- und Kalzium-Ionen in die Zelle strömen. Zur Modulation dieses Kanals gibt es daher zwei Ansätze. Antagonisten blockieren den Kanal, sodass ein Ioneneinstrom verhindert wird und Agonisten ermöglichen über die Aktivierung eine kühlende Wirkung (35). Selektive Liganden werden für die Behandlung von neuropathischen Schmerzen diskutiert (36).

Innerhalb des Entzündungsprozesses spielt der TRPA1-Kanal eine Schlüsselrolle und da diverse kleine Moleküle aus Wasabi, Meerrettich und Knoblauch ihn aktivieren können, ist dieser Kanal ein interessantes Ziel für die Densensibilisierung (37).

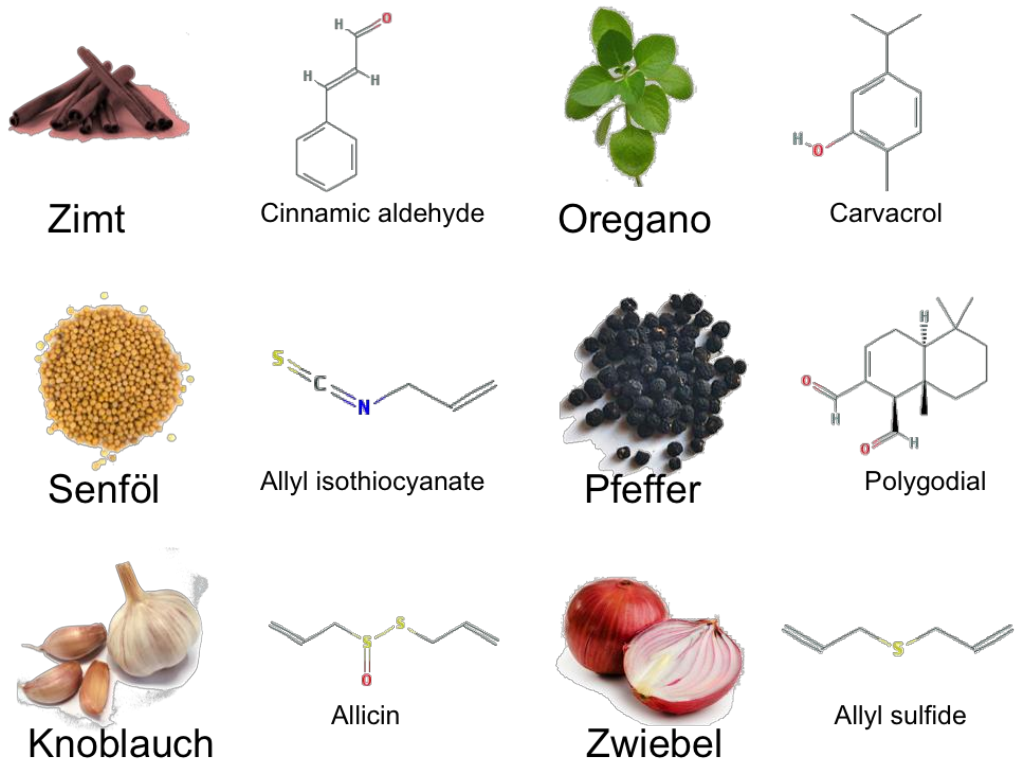

Abbildung 2: **Modulatoren von TRPA1.** Die hier gezeigten Verbindungen aus bekannten Nahrungsbestandteilen sind experimentell bestätigte Modulatoren des TRPA1-Kanals. Damit haben sie potentiell Einfluss auf die Schmerzperzeption und/oder -modulation.

Mit dem TRPA1-Kanal werden die Schmerzen bei überempfindlichen Zahnhälsen assoziiert. Ein häufig angewendetes und sehr wirksames Präparat zur Behandlung der schmerzempfindlichen Zähne beinhaltet Glutaraldehyd (38). Da es sich bei Glutaraldehyd um eine giftige Substanz handelt, wäre eine lokale Applikation von anderen nicht-toxischen Verbindungen, wie beispielsweise Oregano (s. Abbildung 2) sehr interessant. Hierzu gibt es ein Anschlussprojekt, bei dem über unterschiedliche Ansätze nach ähnlichen Substanzen gesucht wird. Die Abbildung 2 zeigt neben Oregano weitere bekannte Aktivatoren von TRPA1.

Auch an Kanälen wie TREK1, TRESK, hERG, ASIC P2X und spannungsabhängige Natrium-Kanäle wird intensiv geforscht, sodass eine umfassende Datenbank mit experimentell bestätigten und potentiellen Liganden der Schmerz assoziierten Targets wertvoll ist. Die erstellte online frei verfügbare Datenbank SuperPain

beinhaltet ca. 8.700 Liganden, die mit Bindungsaffinitäten experimentell bestätigt sind und zusätzlich 100.000 potentielle Liganden (39). Darüber hinaus bietet SuperPain 3D-Modelle der Ionenkanäle mit vorhergesagten Bindungsstellen. Innerhalb der Datenbank können zum einen umfangreiche Informationen über die verschiedenen Ionenkanäle und ihre Wirkmechanismen ausgelesen werden und zum anderen gibt es diverse Suchfunktionen.

Es ist möglich, nach bestimmten Liganden oder Zielmolekülen zu suchen. Auf dieser Basis können dann potentielle Liganden über eine Ähnlichkeitssuche herausgesucht werden. Die Ähnlichkeiten, ausgedrückt als Tanimoto-Koeffizienten, können in einzelnen Heat-Maps, die die Substanzen untereinander vergleichen, dargestellt werden. Für 6.000 Liganden wurden die möglichen Bindungsstellen errechnet, die man sich in den 3D-Modellen komfortabel anzeigen lassen und exportieren kann. Neben umfangreichen chemischen Informationen und Affinitäten der einzelnen Liganden wird auch die Verfügbarkeit bei kommerziellen Anbietern der jeweiligen Verbindungen angezeigt. In den letzten Jahren wurden auf diese Art einige potentielle Verbindungen gefunden, die bekannte Schmerzmedikamente aufgrund von geringeren Nebenwirkungen, geringerer Toxizität oder geringerem Abhängigkeitsrisiko ablösen könnten. Mambalgin, ein Peptid aus dem Gift der Schwarzen Mamba, konnte bei Mäusen eine vergleichbare Schmerzsuppression wie Morphium erzielen – jedoch mit deutlich geringeren Nebenwirkungen, sodass die säuresensitiven ASIC-Kanäle interessante Zielmoleküle in der Schmerzbehandlung zur Vermeidung von Suchtproblemen werden könnten (40). Durch die intensive Forschung an der Kristallstruktur diverser Ionenkanäle ergeben sich laufend weitere Zielmoleküle zur Bekämpfung von Schmerzen (41). Die SuperPain Datenbank stellt einen guten Ausgangspunkt für umfangreiche Schmerzforschungsprojekte und die Entwicklung neuer Therapeutika dar.

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SuperPain—a resource on pain-relieving compounds targeting ion channels

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ABSTRACT

Pain is more than an unpleasant sensory experience associated with actual or potential tissue damage: it is the most common reason for physician consultation and often dramatically affects quality of life. The management of pain is often difficult and new targets are required for more effective and specific treatment. SuperPain (http://bioinformatics.charite.de/superpain/) is freely available database for pain-stimulating and pain-relieving compounds, which bind or potentially bind to ion channels that are involved in the transmission of pain signals to the central nervous system, such as TRPV1, TRPM8, TRPA1, TREK1, TRESK, hERG, ASIC, P2X and voltage-gated sodium channels. The database consists of ~8700 ligands, which are characterized by experimentally measured binding affinities. Additionally, 100 000 putative ligands are included. Moreover, the database provides 3D structures of receptors and predicted ligand-binding poses. These binding poses and a structural classification scheme provide hints for the design of new analgesic compounds. A user-friendly graphical interface allows similarity searching, visualization of ligands docked into the receptor, etc.

INTRODUCTION

Ion channels are proteins forming a pore that allows the flow of ions across membranes. Ion channels are voltage or ligand gated. Some of these proteins help nerve cells to transmit pain signals to the central nervous system and are therefore promising targets for the development of pain therapeutics.

Transient receptor potential channels (TRPs) are a family of 28 human cellular ion channels, varying in homology to each other but all with six transmembrane regions in common, and are nonselectively permeable to cations. There are seven subfamilies that can be divided into two groups. Group 1 includes TRPC, TRPV, TRPA, TRPM, TRPN, and group 2 comprises TRPP and TRPML (1). Compounds perceived as hot stimulate the vanilloid receptor (TRPV). At the same time, this receptor plays a crucial role in pain mediation and is therefore an interesting drug target. Known pungent chemicals with high receptor affinity such as capsaicin or resiniferatoxin were lead structures in drug development toward desensitization (2). Recent research focuses on novel analgesic mechanisms like positive allosteric modulation (3) and broad-spectrum TRP antagonists (4).

The mechanism of cold-induced analgesia was unclear until the discovery of TRPM8. This receptor is also known as the cold or menthol receptor (5). It is activated by cold temperatures and cooling agents, such as menthol or icilin, allowing the entry of Na^+ and Ca^{2+} to the cell (6). Two modulating mechanisms are generally discussed. Whereas antagonists physically block the receptor for cold and menthol, agonists activate TRPM8 and generate a cooling sensation. Selective ligands could be used as a new generation of analgesic drugs in neuropathic pain (7,8).

TRPA1 plays a key role in chemical sensing in the inflammatory pain pathway. Many small molecules, including ingredients like wasabi, horseradish, garlic and mustard oil, can activate the channel. Recently, it has been shown that desensitizing TRPA1 could help in the treatment of neuropathic pain (9,10).

The human Ether-à-go-go Related Gene (hERG) channel or KCNH2 is a voltage-gated potassium channel. It has been the focus of pharmaceutical research for years because the inhibition of hERG potassium channels by drugs can lead to cardiac arrhythmia (11). In 2010, Stary and colleagues published a homology model (12) and a group from Canada investigated structural mechanisms of state-dependent

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drug binding (13). The different binding sites for ions and compounds are defined and the International Conference of Harmonization set up guidelines for drug development. Therefore, new screening methods for the prediction of drug liability to the hERG channel have been developed (14).

Voltage-gated sodium channels are activated through action potential firing. They represent the target for local anesthetic agents. It is challenging to find selective inhibitors of sodium channels in the pain pathway. Genotyping of families suffering from congenital indifference to pain identified mutations in gene coding for Nav1.7 channels (15). A lot of research is performed to find specific blockers to the treatment of pain and epilepsy (16).

There are some complementary resources on ion channels. Multiple analysis for voltage-gated potassium channels from different species is compiled in the voltage-gated potassium channel database VKCDB (17). Protein–protein interactions (PPIs) are the focus of the TRIP database (18): Shin and colleagues manually curated 653 PPIs for mammalian TRP channels. MoleOnline 2.0 (19) is a web server that provides interactive channel analysis to identify active sites.

The International Union of Basic and Clinical Pharmacology (IUPHAR) database provides information on human and rodent receptors (20).

There is a need for a specific resource for pain-relieving compounds targeting ion channels and their 3D homology models so researchers can identify targets and putative ligands.

MATERIALS AND METHODS

Here, we shortly describe our methods used. If you are interested in further details, please read the 'About' page on our Web site.

Compounds

To identify pain-related receptors and ligands, scientific literature was screened via text mining and subsequent manual evaluation. Therefore, 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, written in Java, dynamically queries the indexed data and results in a structured query language (SQL) file containing the textmining hits. An example for a query for the literature search is as follows: [lidocaine (TI) AND ic50 (TI)] OR [lidocaine (abstract) AND ic50 (abstract)]. Found hits were put in a preliminary database and are displayed with keywords highlighted in different colors. In the manual evaluation process, confirmed experimentally determined affinities were moved to the final database. In some cases, the full text of the article was checked. Further information regarding the molecules was retrieved from the PubChem database (21). PubChem is a freely available database, which is provided by the National Center for Biotechnology Information (NCBI). It contains detailed information on ~30 million compounds. All those compounds and additional information were put in a MySQL database. Additional affinities were found via a search in BindingDB (22). BindingDB is a database on experimentally determined protein–ligand interactions. It provides ~782 000 affinities for 6500 protein targets.

Compound clustering

The experimentally determined ligands had to be clustered regarding their similarity to each other by means of a K-means algorithm. As in the K-means algorithm, the number of clusters has to be defined the algorithm was slightly modified. To ensure that the most similar compounds are members of one cluster, a neighbor-joining algorithm was used. The R package with heatmap.2 was used to display the compound similarities in a heatmap.

In silico screening

Putative ligands were indentified through a similarity search. Therefore, the structural fingerprints of each ligand were calculated and used for a similarity search within the PubChem Compound database. The similarity between experimentally determined ligands and potential ligands was calculated with the Tanimoto coefficient. Depending on the chemical topological properties it gives values between zero (no similarity) and one (identical). Compounds with a coefficient >0.90 were classified as putative ligands and included in the database.

Receptor structure

Currently, no radiographic crystallographic structures are available for most of the receptors, but there is some information on the active sites of some of the molecules. To perform dockings a homology model for TRPV1 had to be created. The template structure for the homology modeling of TRPV1 and the models of P2X and ASIC were retrieved from the Protein Data Bank (PDB). The PDB is a freely available database on 3D structures of proteins und nucleic acids (23,24). Models are downloadable and provide coordinates of each atom within the molecule. A homology model of the hERG channel was retrieved from a research group in Germany (12).

Docking

To obtain an understanding of binding mechanisms, the homology model of TRPV1 and the models of P2X, ASIC and hERG were imported into Accelrys Discovery Studio (25). About 6000 ligands were docked into the binding sites with the integrated Docking Tool LibDock. LibDock is a high-throughput docking algorithm. Based on polar and apolar interaction sites up to 1000 ligand conformations were positioned in the binding site. The five best-ranked poses regarding energetic conditions are displayed on the Web site. 'JSmol' was used to implement a molecular viewer, which is JavaScript based.

Web site

The Web site is based on PHP (http://www.php.net/); web access is enabled through Apache HTTP Server (http://httpd.apache.org/). We recommend a recent version of Mozilla Firefox or Google Chrome; alternative browsers like Microsoft Internet Explorer and Apple Safari were tested with some configurations. JavaScript must be enabled, as it is required for all features of the site.

RESULTS

Compound database

A total of 100 000 putative channel modulators were included of which 12 000 are purchasable and 8700 ligands have measured binding affinities.

Compound clustering

A structural clustering of the 8700 compounds with an internal similarity (Tanimoto) above 0.7 was performed and resulted in clusters with member sizes up to 52 compounds. For a better visualization, the member size was limited to 30. The neighbor-joining algorithm resulted in 684 clusters. The similarities are displayed in interactive heatmaps.

Receptor structure

The homology model of TRPV1 was based on the known structure of the Kv1.2 potassium channel, which exhibits significant similarity and a high resolution (2.4 •). The structure was downloaded in PDB format (PDB-ID: 2R9R). Homology modeling was carried out in accordance with Fernandez-Ballester et al. (26) using the SWISS-MODEL server alignment mode. PyMOL was used to create a tetramer and manual refinement was carried out with Accelrys Discovery Studio.

Docking

The docking of 6000 TRPV1, P2X, ASIC and hERG ligands in >1000 different binding poses revealed five binding poses for each ligand. Figure 1 shows capsaicin in the binding site of TRPV1 in an active interactive view. These binding poses were chosen regarding energetic conditions. The results are embedded into the database. Each ligand can be displayed in different manners, as well as the receptor itself (ball and stick, spacefill, etc.). Holding the left mouse button while moving the mouse leads to a rotation. A right click brings up a drop-down menu for zooming, measurements, color adjustments, etc. Safari or Tablet users can use their common gestures to navigate through the docking results. Each binding pose can be exported as a graphic (PNG) or file (MOL).

Usage of the database

There are different ways to use the database and browse through the data. Figure 2 summarizes the main functionalities of the Web site.

'Receptor' holds information on pain-related ion channels. The homology model of TRPV1 and docking results can be found there.

There are two search boxes in the 'Compounds' section. Specific compounds can be found through a property search by typing in the name, PubChem ID, IUPAC or SMILES. It is also possible to search for a specific target such as TRPV1 or hERG or to select certain features like purchasability, IC50, EC50, molweight, rotatable bonds, etc. On the results page, information on compounds including vendors is listed in a table. This table can be used as a starting point for a similarity search or to

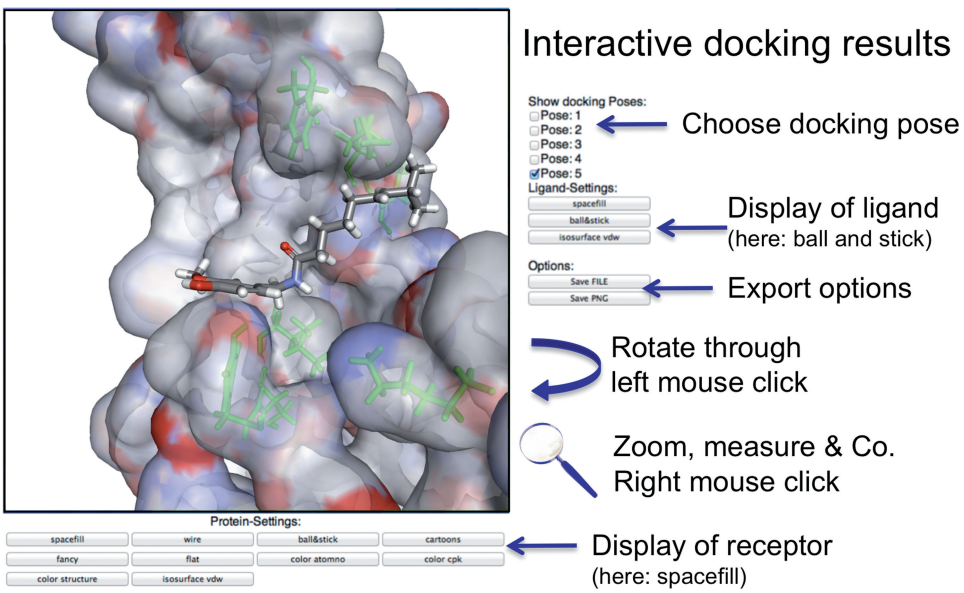

Figure 1. Capsaicin in TRPV1. Capsaicin was put into the homology model of TRPV1 using a high-throughput docking algorithm. Each docked ligand is shown in the interactive view.

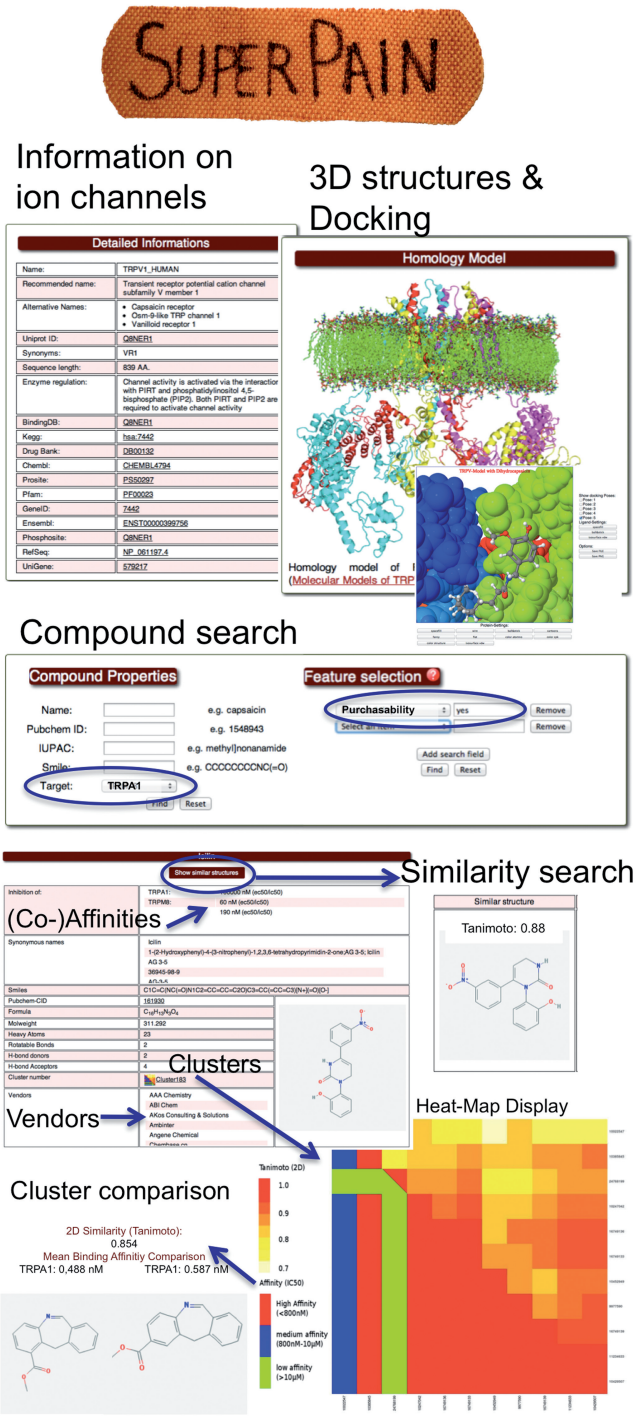

Figure 2. Main functionalities of 'SuperPain'. The database provides information on pain-related ion channels, as well as 3D structures and interactive docking results. The 'Compound' search enables users to find ligands regarding properties or features (name, SMILES, target, IC_{50}, molweight, purchasability, etc.). The 'Results' page allows to perform similarity searches or to view docking results or to see heatmaps with similar structures to compare Tanimoto coefficient or affinities.

compare structures directly by viewing the compound clusters. In the 'Cluster' section all clusters are listed with one compound of the cluster. A search box enables quick access to a distinct cluster. Clicking on a compound also enables browsing through different clusters. The heatmaps are diagonally divided to compare similarity (Tanimoto) and affinity (IC50). Mouseover shows the structure of the compared compounds. Clicking leads to a comparison page with information about (co-) affinities, as well as chemical information and vendors.

DISCUSSION

SuperPain aims at providing a comprehensive resource on ligands for pain-related ion channels.

Acid sensing ion channels (ASICs) belong to the degenerin-epithelial sodium channel superfamily and are voltage independent. They are activated by extracellular acidosis and are involved in different processes, such as taste, mechanosensation and nociception (27). Recently, it has been shown that mambalgin, a three-finger peptide from the venom of the black mamba, suppresses pain in mice without toxicity and fewer side effects than morphine (28). These findings show that ASICs are promising targets in the treatment of pain, especially to avoid addiction problems (29).

In traditional Chinese medicine, tetramethylprazine, sodium ferrulate and puerarin are used in the therapy of pain. It has been found that these compounds target some P2X receptors. These ligand-gated ion channels open in response to binding of adenosine 5′-triphosphaste (ATP). Advances in radiographic crystallography allow *in silico* ligand docking and P2X receptors are becoming therapeutically important drug targets (30).

Further potassium channels of interest are the TWIK-related spinal cord potassium channel (TRESK) and TREK-1. TRESK is a two-pore domain potassium channel that is mainly expressed in dorsal root and trigeminal ganglia (31). This channel is involved in acute and chronic pain and plays an important role in migraine pathogenesis, which makes it a promising target (32). TREK-1 is an interesting target because of its co-expression with TRPV1 and its involvement in polymodal pain perception (33). The present intensive research on 3D structures will improve the development of channel modulators. For example, there are different binding sites for agonists and antagonists (34). The binding sites of the ion channels are rather large, which allows binding of a variety of compounds if certain physiochemical property conditions are fulfilled. This is reflected by the great diversity of the pain-related compounds. They show a low mean similarity of 0.4 compared with other targets like PARP (0.6). It is also important to find out more about the interactions or co-inhibition of different receptors for pain. In the database, they can be found by choosing 'MultiTarget' from the 'Compound Properties' search box. For example, menthol is known to be an inhibitor of TRPM8 with a half maximal effective concentration (EC_{50}) of 29 000 nM. At the same time, it has also inhibitory potency on TRPA1 with an EC_{50} of 28 400 nM. Cannabigerol is a compound that occurs naturally in hemp strains. In contrast to other cannabinoids, it is nonpsychoactive because it is not only a α_2-adrenergic receptor agonist, but also a mild CB_1 receptor antagonist

(35). Although the compound is not well-studied, it has been found to lower the intraocular pressure (36) and there is some research on treating inflammatory bowel diseases with cannabigerol (37). 'SuperPain' stores affinities for TRPA1, TRPM8 and TRPV1 for the compound. These experimentally determined co-affinities suggest that these multi-target compounds might be promising for the development of pain therapeutics (38).

Availability

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

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2.2 Pharmakokinetik von Xenobiotika

Bei der Entwicklung von Medikamenten liegt der Fokus in der Regel auf deren Pharmakodynamik. Bei der SuperCYP Datenbank wurde der Ansatz verfolgt, den Stoffwechsel, die Pharmakokinetik, in die therapeutischen Entscheidungen einzubeziehen (42). Die Familie der Cytochrom P450 Enzyme (CYPs) wird seit Jahrzehnten umfangreich beforscht, da der Metabolismus der meisten Medikamente und Xenobiotika durch CYPs realisiert wird.

Da manche Medikamente inhibitorische oder induktive Effekte auf bestimmte CYPs ausüben, sollte die Kombination und Dosierung mehrerer Medikamente immer unter Berücksichtigung des gleichzeitigen Stoffwechsels erfolgen. Patienten mit rheumatoider Arthritis stehen häufig unter medikamentöser Dauertherapie. Aggressive Parodontitiden müssen mitunter mit systemischen Antibiotika behandelt werden. Ungünstige Medikamentenkombinationen können dann mitunter zur ineffektiven Therapie beider Erkrankungen führen (Tabelle 1).

Medikament	**Gruppe**	**1A2**	**2C8**	**2C9**	**2C19**	**3A4**
ASS	NSAID		S	S	Ind	
Amoxicillin	Antibiotikum				S	
Ciprofloxacin	Antibiotikum	Inh				Inh

Tabelle 1: **Ineffektive Therapie durch Enzyminduktion.** S: Substrat, Ind: Induktor, Inh: Inhibitor. Die Tabelle 1 zeigt eine ungünstige Kombination zweier Medikamente. ASS wirkt induktiv auf CYP 2C19 das Amoxicillin abbaut. Um den gleichen therapeutischen Effekt zu erzielen, müsste die Dosis von Amoxicillin erhöht werden, denn dieses Medikament wird in Kombination mit ASS schneller eliminiert als in einer Monotherapie. Eine sinnvolle Alternative zu Amoxicillin wäre zum Beispiel das gezeigte Ciprofloxacin, das einen anderen Stoffwechselweg durchläuft.

Mit Kenntnis dieser Zusammenhänge können unerwünschte Nebenwirkungen vermieden werden und bei geschickter Kombination unterschiedlicher Medikamente zusätzlich auch Medikamentendosierungen optimiert werden.

Mittelfristig wird es möglich sein, nicht nur qualitative Empfehlungen zu geben, sondern durch pharmakokinetische Ansätze werden konkrete Dosisanpassungen bei

bestimmten SNPs oder Medikamentenkombinationen zugänglich. Die mathematischen Methoden dafür existieren bereits (Multi-Kompartment-Modelle; Differentialgleichungssysteme), allerdings sind kombinierte Genotypisierungen und Plasma-Level-Bestimmungen noch nicht im klinischen Alltag angekommen, sodass nicht genügend Daten vorliegen, um die Differentialgleichungen verlässlich zu parametrisieren. Für einzelne Medikamente (z.b. Phosphamide, Statine) wurden entsprechende Studien initiiert, um diese Richtung voranzubringen.

Ziel dieses Forschungsprojekts war es, eine schnell und einfach abrufbare Auflistung möglicher Medikamenteninteraktionen einsehen zu können und dennoch die Möglichkeit zu haben, sich detailliertere Informationen aus der PubMed Datenbank anzuschauen, um zu prüfen, ob die vorgeschlagenen Medikamentenalternativen in Frage kommen. Durch Kombination von Medikamenten, die über unterschiedliche CYPs abgebaut werden, können Unwirksamkeit oder unerwünschte Nebenwirkungen verhindert werden.

Hierfür wurden Informationen über CYPs aus der wissenschaftlichen Literatur und verschiedenen Web-Ressourcen extrahiert. Mithilfe spezieller Schlüsselwörter und einer automatisierten Suche wurde PubMed nach relevanten Artikeln durchsucht. Gleichzeitig wurde nach WHO-Medikamenten und deren Synonymen gesucht. Die gefundenen Publikationen wurden von einem Team von Wissenschaftlern manuell nach Informationen zum Metabolismus durchsucht. Den CYPs wurden diejenigen Medikamente zugeordnet, die im Metabolismus eine Rolle als Substrat, Induktor oder Inhibitor spielen und die zugehörige PubMed-Referenz wurde integriert. Es ist möglich, sowohl nach einzelnen Medikamenten und ihrem Stoffwechsel zu suchen, als auch mehrere Medikamente zusammenzustellen und zu überprüfen, ob und wie diese sich gegenseitig beeinflussen (19).

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Research Article Open Access

Drug Interactions Involving the Cytochrome P450 Enzymes: Analysis of Common Combinations of Antibiotics and Pain Relieving Drugs

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Abstract

Objective: For clinicians it is challenging to oversee complex drug interactions of multi-drug administration. Rheumatoid arthritis (RA) patients are frequently under long-term medication with multiple anti-inflammatory and pain-relieving drugs, which are mainly metabolized by the Cytochrome P450 enzymes (CYPs). Additionally, treatment of co-morbidities, such as inflammatory periodontal disease (PD) may have to involve further drug administration. The aim of this investigation was to analyze drug interactions in the therapy of RA and PD and to provide a resource for health professionals to easily check interactions and avoid potential side effects.

Methods: Information on drug administration in the therapy of RA and PD and expression data of human tissues regarding CYPs was gathered and/or analyzed from scientific literature and web resources. A literature compilation was developed and CYP interaction tables were generated.

Results: Side effects, such as enzyme overload or enzyme induction and inhibition may occur in the therapy of RA and PD. To overcome these problems, a web-interface was developed to optimize drug cocktails. The compilation provides manually curated information on the metabolism of 1,500 drugs including 100,000 PubMed references, covering a variety of co-morbidities. Moreover, based on the WHO classification system for drugs (ATC-codes), the knowledge base offers drug alternatives, avoiding CYP-related problems. The web-interface is publicly available: http://bioinformatics.charite.de/perio

Conclusions: After a detailed drug anamnesis, health professionals should use a web-interface to check drug interactions involving CYP metabolism, which may circumvent adverse side effects and optimize interdisciplinary drug therapy.

Introduction

Rheumatoid arthritis (RA) is the most frequent inflammatory joint disease affecting more than 50 million people worldwide [1]. RA patients are frequently treated with pain relieving and anti-inflammatory drugs (NSAIDs). Furthermore, corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs) and biologics are administered depending on RA severity and progression [2]. In 2008, a world-wide group of rheumatologists developed a set of recommendations for the RA treatment, which is updated at regular intervals [3]. The recommendations are target-based on evidence and expert opinion. The primary treatment aim is the clinical disease remission. Also, the individual drug therapy is at least adjusted every three months, which requires frequent drug anamnesis and adaptation by health professionals besides rheumatologists.

The RA etiology is unclear, however next to genetic and environmental factors such as age, gender, HLA genotype and smoking, bacterial infections seem to play an important role [4]. It is proposed that RA results from a failure of the immune response attacking an unknown antigen such as hidden viral or bacterial infections, also diseases preceding RA may cause a failure immune response to viral or bacterial antigens [5].

Periodontal disease (PD) is a bacterial infection affecting the periodontium, which can cause increasing degradation of tooth-supporting soft- and hard tissues, ultimately resulting in tooth loss [6]. Gram-negative anaerobic bacteria, organized as a structured biofilm on the tooth surface are the primary cause involved in the initiation and the progression of PD [7]. The best described periodontal pathogens are *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythensis* and *Porphyromonas gingivalis* (*P. gingivalis*) [8]. *P. gingivalis*, one of the major periodontal pathogens, is able to invade endothelial cells and human chondrocytes [9]. It is the only known bacterium expressing the peptidylarginindeiminase (PAD) enzyme, which is responsible for the post-translation and conversion of arginine to citrulline [5]. Citrulline modifications lead to the production of anti-CCP antibodies, which are found most frequently in RA patients [10]. Furthermore, aggressive periodontitis, affecting young individuals, is characterized by severe periodontal attachment loss and bone destruction. In comparison to adult periodontitis, aggressive periodontitis shows a more rapid disease onset and a faster progression. It was shown that a combination of mechanical and antibiotic treatment effectively provides favorable clinical results on periodontal and systemic health in generalized aggressive periodontitis patients [11]. In general, the selection of the antibiotic is adapted to the spectrum of bacteria (Table 2).

Increasing evidence shows that patients with RA have an increased prevalence of periodontal attachment loss compared to healthy

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individuals [12]. Evidence from epidemiological studies suggests a bi-directional association [13]. In both the diseases, dysregulated immune responses seem the crucial factor facilitating tissue degradation and loss of function [14]. Intervention studies indicate causal relationship by showing that periodontal therapy has beneficial systemic effects on RA disease activity [15]. PD and PA are prevalent chronic inflammatory diseases associated with significant morbidity and mortality and therefore immense impact upon the economy, health and quality of life. RA and PD are associated with increased mortality due to a number of co-morbidities. Both are chronic inflammatory diseases associated with soft and hard tissue damage, a dysregulation of immune response and common genetic and lifestyle factors influencing the diseases [16].

A number of cross-sectional studies reported an increased incidence of PD in RA patients [17] and a higher prevalence for RA in patients suffering from PD. An increased risk for systemic diseases such as cardiovascular disorders, diabetes and osteoporosis was described for both diseases [18]. Therefore, next to the drug-therapy of RA and PD additional drugs may have to be administered for the treatment of the co-morbidities. Therefore, further undesired drug-drug interactions may be admitted by health professionals treating RA patients.

Drug metabolism is a complex biochemical network, which consists of many different parts and reactions in the human organism. Some drugs are excreted in urine and feces without passing any metabolic modifications in the liver. However, most of the systemic drugs have a multi-step metabolism (typically oxidation and conjugation). The oxidation reactions are mainly catalyzed by the Cytochrome P450 enzymes (CYPs) family of CYP enzymes [19], which belong to the family of monooxygenases has been the focus of pharmaceutical research for decades. CYPs catalyze a large amount of chemical reactions, such as alcohol oxidations, dehydrogenation and isomerizations. It is a difficult task of medical science and daily clinical practice to find effective and safe combinations of drugs that do not affect each other's metabolic pathways. If this is not taken into account, severe adverse effects including death occur. The Human Genome Project discovered 57 human CYPs [20]. Due to many polymorphisms and inducability, the biological activities of the CYPs vary noticeable among humans, which is an important issue for researchers as well as clinicians. Knowledge of the level and the catalytic activity of the specific CYP as well as the effect on drug metabolism could and should lead to personalized drug dosages to optimize the therapeutic effect and minimize harmful side effects. If a drug induces a specific CYP, which is also active in another drug's metabolism, the dosage of the first drug should be increased to achieve the same therapeutic effect [21]. In case of a CYP inhibition, the dosage can be reduced, which lowers side effects.

Due to multi-drug administration, adverse side effects, such as deadly acute renal failure (31) are discussed intensely in pharmaceutical research [22].

Frequently occurring problems, which we address here, are firstly adverse side effects because of enzyme overload and secondly, ineffective therapy because of enzyme induction or inhibition. Therefore, drug interactions in the therapy of RA and PD were analyzed in the present study.

Materials and Methods

Textmining

Information on drug metabolism is spread over 100,000 articles in PubMed. To collect relevant articles a specific search tool was developed. Abstracts of PubMed database were automatically filtered for relevant articles using specific keywords. Medical subject headings (MeSH) represent the National Library of Medicine's vocabulary thesaurus and were used for disease definitions and synonyms. The abstracts were screened for WHO-drugs and their synonyms, as well as a set of human CYPs with synonyms and the papers found in PubMed were manually processed. Each drug was attributed to those CYPs that are involved in drug metabolism as a substrate, an inhibitor or an inducer.

Treatment schemes

Information on drug administration in the therapy of RA and PD was collected from scientific literature. Additionally, for RA, international recommendations [23] and for PD different national guidelines [24] could be taken into account. Web resources provided further information on drug metabolism, e.g. Nelsons Homepage [25], Flockharts Interaction table [26], University of Maryland's Drug Checker, PubChem [27], Protein Data Bank [28] and FDA-files.

Drug classification

The recommendations of the WHO Expert Committee for updating the WHO Model List of Essential Medicines are updated annually [29]. In 2004, a list of all items, according to their 5-level Anatomical Therapeutic Chemical (ATC) classification code was published. The ATC-code classifies drugs into different groups according to anatomic site of action, therapeutical effect and chemical structure. The therapeutic subgroup, which is determined by the second level, was used to find drug alternatives.

Expression data

Affymetrics data were used to compare the CYP mRNA expression of human body tissues. The series of datasets taken from GEO (Gene expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) were generated from ten donors and represent normal human bodies (Series GSE3526, [30]). It contains seven different tissues, oral, pharyngeal, esophageal and intestinal mucosa, as well as skeletal tissue and bone. All probe sets related to Cytochromes were normalized and condensed to 40 types of CYPs. To assess differences in expression, a heat-map was built with Genesis [31].

Database and web-server

Two CYP interaction tables were generated for the therapy of RA and PD. Numerous problems, such as enzyme overload or enzyme induction and inhibition could occur in the combined therapy of RA and PD. Some of these drug-drug interactions are rather unnecessary because the choice of another antibiotic could already circumvent the problem. In the present study, a web-interface for clinicians to check drug-drug interactions was generated to overcome CYP based problems. The database provides information on drug metabolism including PubMed references. Based on the WHO classification system (ATC), the database provides drug alternatives.

The present 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 and the web access is enabled via Apache Webserver 2.2.

Results

The results of the present literature analysis are summarized in tables 1 and 2, respectively. Table 1 show that especially CYPs 2C8,

Drug	NSAIDs	DMARDs	Opioids	Steroids	Substrate	Inhibitor	Inducer
Aspirine	X				2C8, 2C9		2C19
Diclofenac	X				2C8, 2C9, 2C19, 3A4,	2C8, 2C9, 3A4	
Ibuprofen	X				2C8, 2C9, 2C19	2C9	
Indometacine	X				2C9, 2C19	2C9, 2C19	
Ketoprofen	X					2C9, 2C10	
Metamizol	X						2B6, 3A4
Naproxen	X				1A2, 2C8, 2C9		
Oxaprozin	X				2C9		
Paracetamol	X				1A1, 1A2, 2A6, 2C8, 2C9, 2D6, 2E1, 3A4	3A4	2D6, 2E1, 3A4/5
Phenylbutazon	X				2C9		3A4
Piroxicam	X				2C9	2C9	
Betamethasone				X	19A	3A4	19A
Hydrocortisone				X	3A4	3A4	2C8, 3A4
Prednisone				X	3A4	3A4	1A1, 1A2, 3A4
Codeine			X		2D6, 3A4	2D6	
Fentanyl			X		3A4	3A4	
Morphin			X		2C8, 3A4		
Tramadol			X		2B6, 2D6, 3A4	2D6, 3A4	
Chloroquine		X			1A1, 2C8, 2D6, 3A4	2D6	
Cyclosporine		X			3A4	2C19, 2D6, 3A4	
Hydochloroquine		X				2D6	
Leflunomide		X			2C8, 2C9	2C8, 2C9	

Additional administration of antibiotics in the therapy of PD could influence the metabolism of the other drugs administered for RA therapy. Potent antibiotic agents against periodontal bacterial pathogens are listed in Table

Table 1: Drugs in the therapy of RA with CYP metabolism. Involved CYPs are ordered in mode of action (substrate, inhibitor, inducer) and references are given in supplementary material.

	Bacteria					
	Aa	Tf	Pg	Substrate	Inhibitor	Inducer
Amoxicillin	+	+	++	2C19		
Ciprofloxacine	+				1A2, 3A4	2E1
Clindamycine		++		3A4	3A4	
Doxycycline		+			3A4	
Metronidazole	++		+	2C9, 3A4	2C9, 3A4	
Tetracycline	+	+			3A4	

Table 2: Effectiveness and CYP metabolism of antibiotic agents used in the therapy of PD. References given in parentheses. "Aa" means Aggregatibacter actinomycetemcomitans, "Tf" Tannerella forsythensis and "Pg" Porphyromonas gingivalis. +: 10-fold increased, ++: 10^2-fold increased concentration of antibiotic in gingival fluid, expressed in multiples of in-vitro measured minimal inhibitory concentration [32].

2C9, 2C19, 2D6 and 3A4 are involved in the metabolism of the analyzed drugs used for treatment of RA and PD [32].

Expression data

The built heat-map lists seven tissues involved in RA and PD and the expression of several CYPs therein (Figure 1). Expression ranges from -2.24-fold lower to 2.24-fold higher values. The CYP expression in target tissues has not been taken into account so far, but is an interesting issue because it is a major factor for the effective retention period. For example, CYP 3A7, which was formerly known as fetal enzyme, was recently shown to be upregulated in the bone [32]. This means that the function of the CYP 3A family is significantly increased, which leads to shorter duration of action of drugs like Paracetamol, Diclofenac, Prednisone, Fentanyl etc.

Discussion

In an aging society with increasing morbidities and co-morbidities drug interactions have to be realized and or prevented by health professionals. One of the most difficult tasks of the decision making process is to find combinations of drugs that do not affect each other's metabolic pathways. Despite the large amount of information on CYPs, optimizing multiple drug prescriptions using CYP metabolism is still complicated [33]. Drug-drug interactions are complex and information on drug metabolism is spread over 100,000 articles in PubMed, which may be overwhelming and not possible to handle by the clinician. Information on CYP-structures [34], binding sites [35], interactions and different genotypes [36] must be combined to allow reducing side effects and to determine correct dosages of medicine undesired side effects when prescribing more than one drug [37]. To overcome this problem a tool for medical and dental clinicians was generated to identify and examine drug-drug interactions online. The SuperCYP database [38] contains information on 1,170 drugs with more than 3,800 interactions including scientific references. This comprehensive resource is freely available at http://bioinformatics.charite.de/perio and is also usable on smartphones and tablet-PCs and could be used as basis for personalized medicine.

Evidence of an association between RA and PD, two of the most common inflammatory diseases in human, is increasing [39].

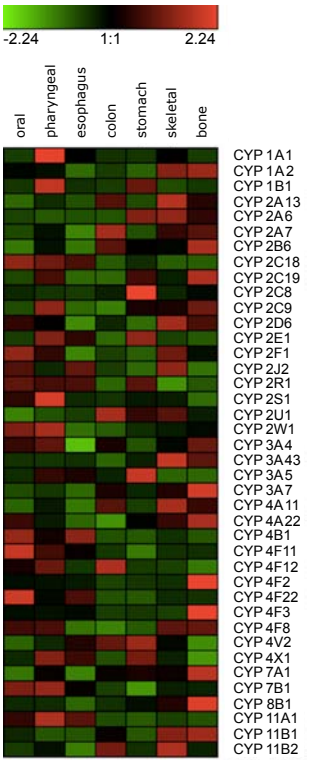

Figure 1: Heat-map of target tissues in RA and PD and the expression of several CYPs therin. Expression ranges from -2.24-fold lower (green rectangles) to 2.24-fold higher values (red recatangles).

In addition, both diseases are associated with systemic chronic inflammatory co-morbidities such as cardiovascular disease. Based on the fact that the medication of pain relieving and disease-modifying drugs can hardly be modified, it is primarily the dentist's task to choose an antimicrobial agent for adjunctive periodontal treatment that is on the one hand most effective in its antibacterial efficacy and on the other hand does not negatively affect the therapy and its side effects in RA patients.

The present data on CYP metabolism suggests two key problems of drug-drug interactions in the treatment of PD in RA patients, discussed below (Tables 3 and 4). First, Aspirine, a commonly used NSAID in the therapy of RA, is metabolized by CYP 2C8 and 2C9 and induces 2C19, which is also the substrate of Amoxicillin, a ß-lactam antiobiotic drug often prescribed as antimicrobial therapy adjunctive to mechanical debridement in oral infections such as aggressive periodontitis. Due to induction of CYP 2C19 and inactivation of Amoxicillin may be possible. Therefore, a replacement by another group of antibiotic agent, such as Ciprofloxacine, which is also effective against periodontal pathogens, would be less harmful with respect to CYP metabolism, and therefore could easily bypass this problem (Table 3).

The table shows drug interactions of the NSAID, Aspirine, with antimicrobial drugs, Amoxicilline (red line because of the conflict regarding CYP 2C19 [orange cells]) and Ciprofloxacine. Ciprofloxacine avoids the CYP 2C19 conflict (green). "S" means substrate, "Ind" means inducer and "Inh" means inhibitor. Suggestions like that are automatically generated by the Web-Server using the classification and metabolic information stored on the server for the drug-cocktail entered by the user.

In addition, in the therapy of RA, NSAIDs and DMARDs are often combined with each other and drug-drug interactions often occur. If an antibiotic agent with the same metabolic pathway is administered, side effects because of enzyme overload are possible and could be avoided by choosing agents with different metabolic pathways. The NSAID, Oxaprozine, and the Leflunomide, a DMARD, share the same metabolic pathway via CYP 2C9. Additionally, Leflulomide inhibits CYP 2C8 and 2C9. Administration of Amoxicilline in combination with the antimicrobial drug, Metronidazol, which uses the same metabolic pathway as Oxaprozine and Leflulomide and inhibits the CYP, as well, could lead to adverse side effects because of enzyme overload. Clindamycine, which is also potent against periodontal pathogens, might be a good alternative (Table 4) [40,41].

Advances in genetic research have enabled genotyping and analysis of individual data on expression of target genes and metabolic enzymes. Such expression data in target tissues should be considered in selection of drugs.

	Drug	1A2	2C8	2C9	2C19	3A4
Aspirine	NSAID		S	S	Ind	
Amoxicilline	Antibiotic agent				S	
Ciprofloxacine	Antibiotic agent	Inh				Inh

Table 3: Ineffective therapy because of enzyme induction or inhibition.

	Drug	2C8	2C9	2C19	3A4
Oxaprozine	NSAID		S		
Leflunomide	DMARD	S, Inh	S, Inh		
Metronidazol	Antibiotic agent	Inh	S, Inh		S, Inh
Amoxicilline	Antibiotic agent			S	
Clindamycine	Antibiotic agent				S, Inh

Table 4: Potentially adverse side effects because of enzyme overload.

The Web-Server presented in this study provides a user-friendly platform enabling medical and dental health professionals to optimize drug choice and combinations regarding the degree of CYP capacity utilization. With respect to increasing evidence of associations between oral and systemic chronic inflammatory diseases, such as PD and RA, knowledge about drug interactions become crucial to optimize health care.

Conflict of interest

There is no actual or potential conflict of interest.

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Authorship Contributions

Participated in research design: Preissner, Kuzman, Pischon

Performed data analysis: Preisser, Kuzman

Wrote manuscrcript: Preissner, Pischon

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2.3 Personalisierte Schmerzmedizin

Immer mehr Daten zur genetischen Diversität bzw. ‚personal genomes' werden bekannt. Für die CYPs wurden bereits weit über 2.000 Mutationen beschrieben und nicht wenige davon haben einen entscheidenden Einfluss auf die Funktionalität des Enzyms. Patienten mit akutem Koronarsyndrom erhalten in der Regel Thrombozytenaggregationshemmer (ASS und Clopidogrel) zur Reduktion von ischämischen Ereignissen (43,44). Allerdings sprechen 29% der Patienten nur schwach auf Clopidogrel an und daher ein erhöhtes Risiko für erneute ischämische Ereignisse besteht (45). Hierfür werden unterschiedliche Gründe genannt (geringe Compliance, hoher Body Mass Index, Diabetes mellitus) (46), allerdings handelt es sich bei Clopidogrel auch um ein so genanntes Prodrug, das erst durch CYP-Aktivierung wirksam wird. Es konnte auch gezeigt werden, dass der Polymorphismus auf CYP 2C19*2 zu einem 30% erhöhten Risiko für kardiovaskuläre Ereignisse bei der Behandlung mit Clopidogrel führt (47-49), wobei noch weitere Polymorphismen mit der Clopidogrel-Resistenz in Verbindung gebracht werden konnten (50). Die Brücke zwischen den bekannten Daten und therapeutischen Konsequenzen fehlt allerdings bisher weitgehend. Ziel dieses Projektes war es, die häufigsten und für den Medikamentenmetabolismus relevantesten CYP-Polymorphismen in der kaukasischen Bevölkerung herauszufinden (51). Damit kann die Entwicklung von Tests für eine effiziente Genotypisierung vorangebracht werden, was zu einer besseren, personalisierten medikamentösen Therapie führen könnte.

Für das am stärksten polymorphe CYP 2D6 konnten 114 Einzelnukleotid-Polymorphismen (SNPs) gefunden werden und für acht weitere wichtige CYPs jeweils mehr als 22 SNPs. Abbildung 3 zeigt die Anzahl der gefundenen SNPs pro CYP.

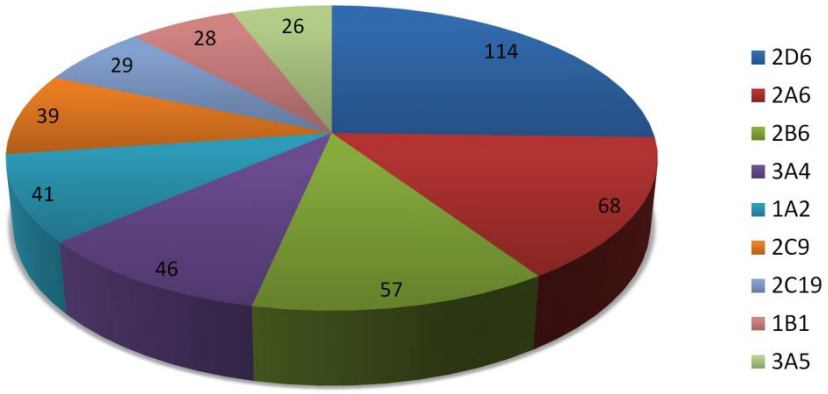

Abbildung 3: **Anzahl bekannter SNPs pro CYP.** Das Diagramm illustriert die Anzahl der bekannten Polymorphismen in den verschiedenen CYPs.

Die gefundenen Varianten wurden nach Häufigkeit in der kaukasischen Bevölkerung sortiert und nach Relevanz für den Medikamentenmetabolismus sortiert, denn nicht alle CYPs haben signifikanten Einfluss darauf. Mithilfe der SuperCYP Datenbank konnte gezeigt werden, das zwölf CYPs für 93% des Medikamentenstoffwechsels verantwortlich sind und allein die hoch-polymorphen CYPs 1A2, 2D6, 2C9 und 2C19 für 40% aller metabolisierenden Reaktionen verantwortlich sind. Dies zeigt deutlich die Notwendigkeit der Genotypisierung bei der Verabreichung von Medikamenten, die über diese CYPs metabolisiert werden. Ein weiteres interessantes Resultat der Untersuchung war die Auswertung von CYP-Expressionsdaten in unterschiedlichen Geweben. So werden die CYPs 2F1, 4B1, 4F8, 11S, 11A, 11B1, 11B2, 19 und 24 nicht in der Leber exprimiert und 39 CYP-Isoformen zeigten in mindestens einem Gewebe eine höhere Expression (52). Das CYP 2C8 zeigt beispielsweise in der Lunge eine 5-fach höhere Expression, CYP 2B6 eine 4-fach höhere Expression in den Nieren und CYP 2C18 konnte in der Mundschleimhaut und dem oberen Gastrointestinaltrakt vermehrt nachgewiesen werden. Die unterschiedliche Verteilung der CYPs in den Geweben könnte einen Einfluss auf spezifische Nebenwirkungen von Medikamenten haben. Bei der Behandlung mit Cyclophosphamid (CPA) kann es zu einer Hyponatriämie kommen. CPA ist ein Prodrug, das durch CYP 2B6 in seine aktive Form umgewandelt wird (53). Die Hyponatriämie ist das Ergebnis einer CPA-induzierten hohen Expression der Aquaporine 1 und 7 (54). Da CYP 2B6 vermehrt in

den Nieren exprimiert wird, führt eine höhere Konzentration von aktivem Cyclophosphamid hier zu dieser unerwünschten Nebenwirkung. Außerdem könnten Medikamente in den Zielgeweben metabolisiert werden, wodurch sie wiederum auch unwirksam werden könnten.

Insgesamt konnte die Studie vier CYPs (1A2, 2D6, 2C9 und 2C19) und 34 polymorphe Allele detektieren, die einen signifikanten Einfluss auf den Medikamentenmetabolismus in der kaukasischen Bevölkerung haben. Wenn individuelle genomische Analysen zur klinischen Routine gehören, können diese Daten für die Vorhersage von Komplikationen bei der medikamentösen Therapie verwendet werden. Dieser vielversprechende Ansatz wird aktuell in Kooperation mit der pädiatrischen Onkologie der Charité für Patienen mit Rezidiven von akuter lymphatischer Leukämie verfolgt.

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].

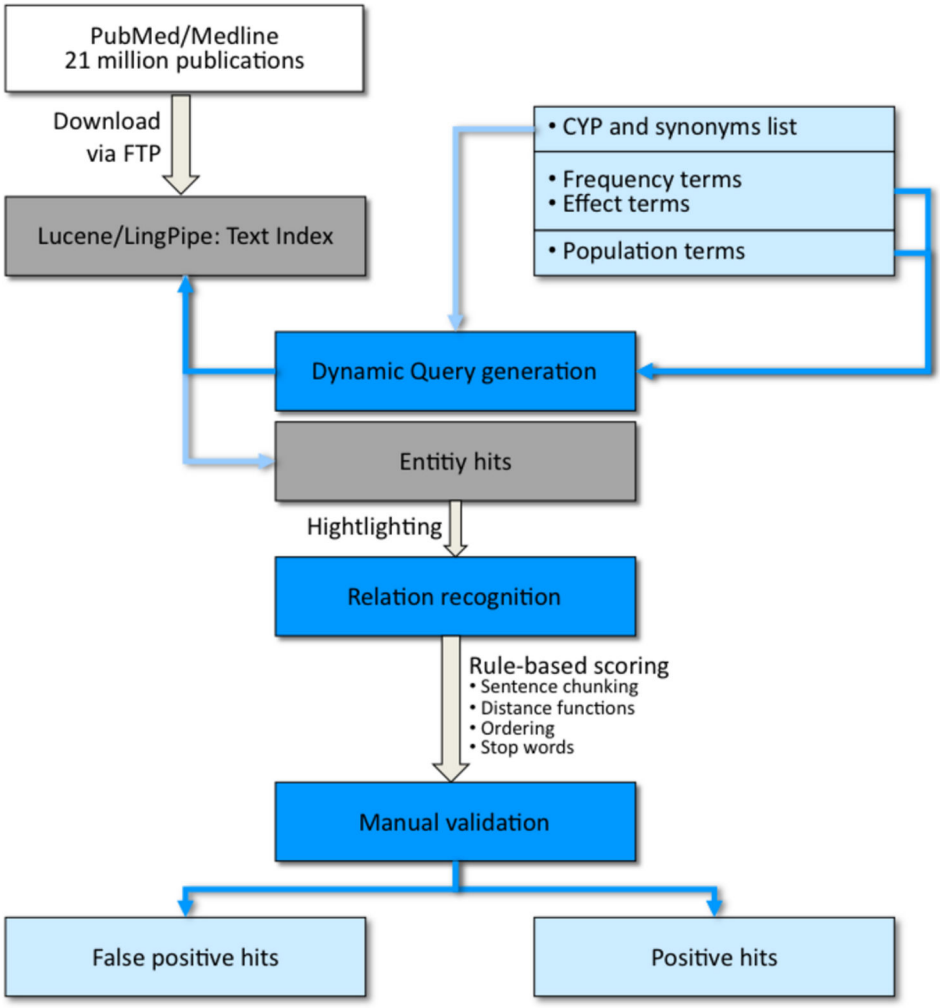

Figure 1. Flow-chart of the methods used for the text mining approach.

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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.

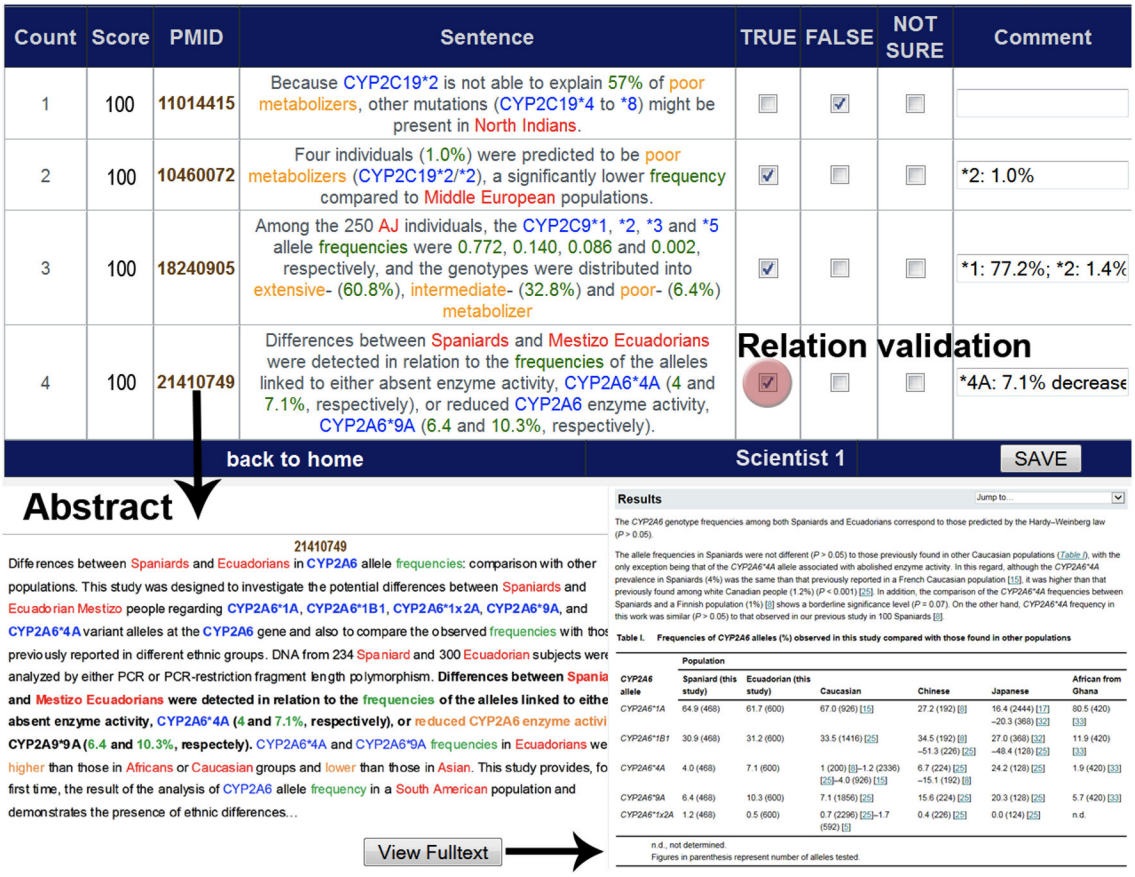

Figure 2. Text mining validation tool. The table shows the text mining validation tool with columns for score, PubMedID, relation sentence and checkboxes for the validation. The SNPs are highlighted in blue, frequencies in green, effects in orange and the ethnicities in red. 'Scientist 1' reads the abstract 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

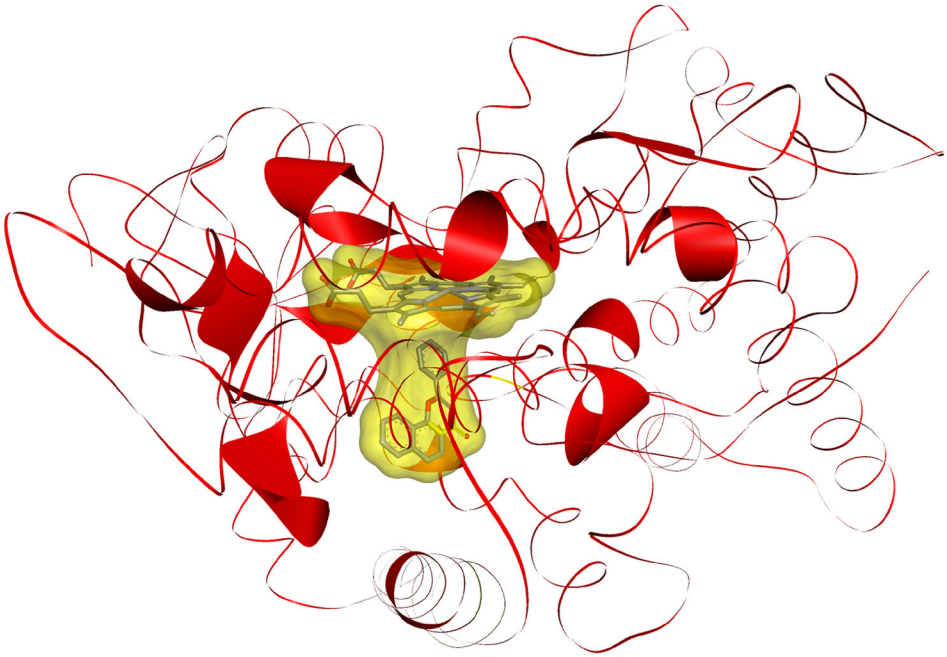

Figure 3. 3D structure of a cytochrome with heme and a ligand with localization of frequent SNPs. Frequent SNPs in the four most polymorphic CYPs (1A2, 2C9, 2C19 and 2D6) were labeled in this 3D model of CYP 2D6 (PDB ID: 3TDA). Therefore, the ribbon was enlarged in the appropriate positions. The binding-side (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

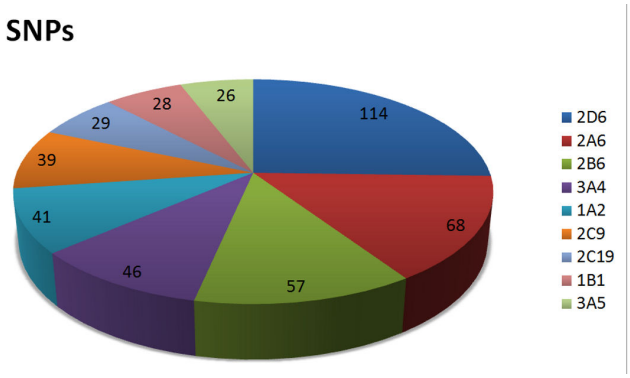

Figure 4. Number of known SNPs per CYP. The pie chart illustrates the number of known SNPs in different CYPs.
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cortex, which possessed 6-fold higher expression of CYP 11A1, 11B1 and 11B2 (compared to the mean expression). Interestingly, no other tissue 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

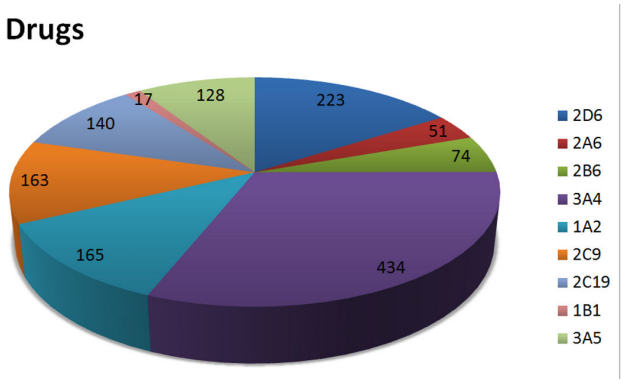

Figure 5. Number of drugs metabolized per CYP. The pie chart illustrates the number of drugs that can be metabolized by a specific CYP.

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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

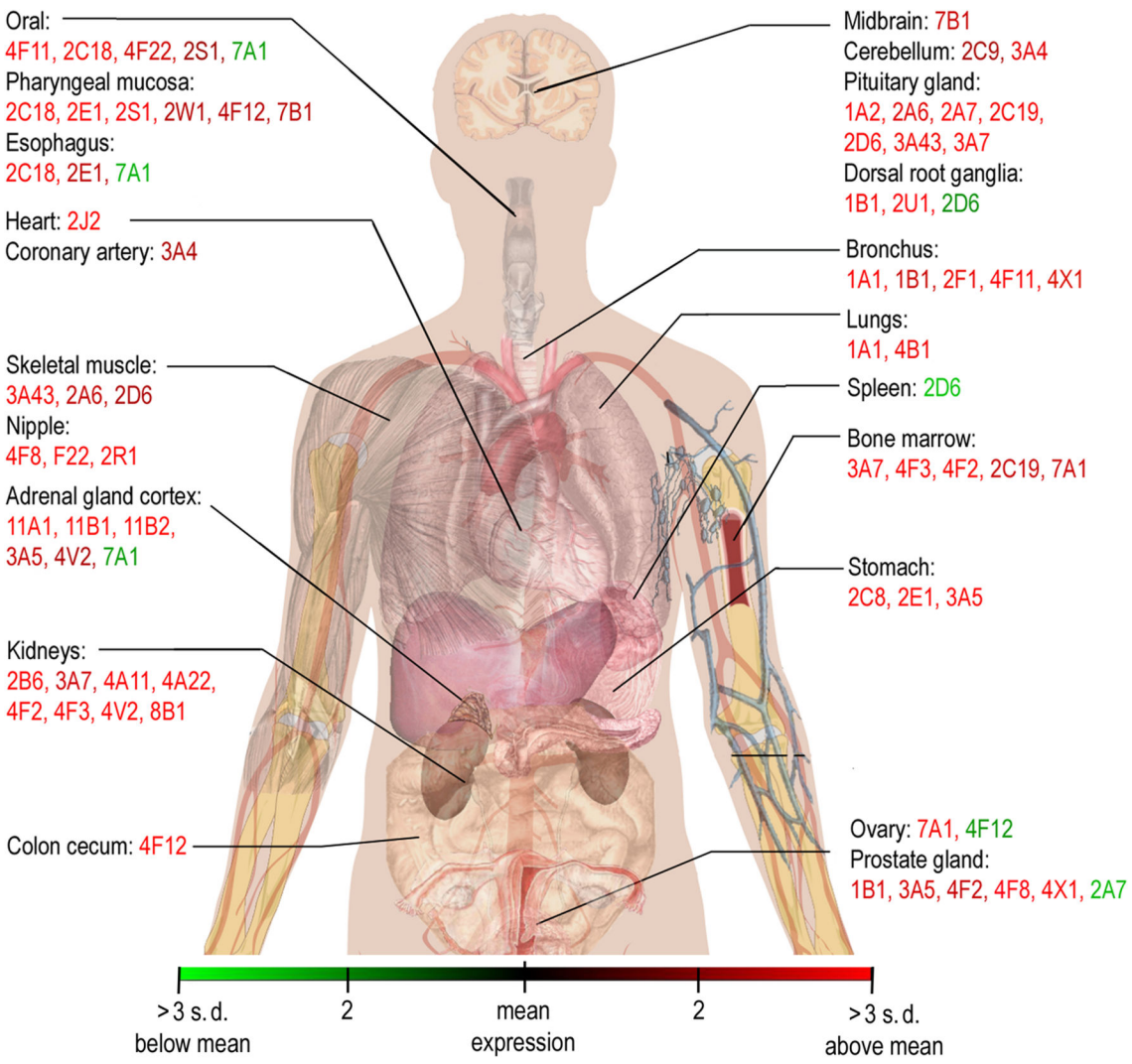

Figure 6. Body map of cytochrome P450 enzyme expression. A schematic map of the specific expression of different CYPs in human organs is presented. Expression values are relative to the mean expression in all organs. At least three-fold higher expression of a CYP in one organ is indicated by red text. 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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2.4 Stoffwechsel und Nahrungsmittelinteraktionen von Medikamenten

Neben den CYPs, die in der Regel durch Oxidationsreaktionen reaktive Gruppen generieren (Phase I), gibt es noch weitere wichtige Enzyme im Medikamentenstoffwechsel. Phase II Enzyme nutzen diese reaktiven Gruppen um die Polarität und somit Wasserlöslichkeit zu erhöhen (55). Transporter (Phase III) sind wichtig, um die Migration von hydrophilen Molekülen über verschiedene Barrieren zu ermöglichen. Inzwischen konnte gezeigt werden, dass die Mehrzahl der Medikamente die Zelle durch mindestens einen Transporter erreicht (56). Der Metabolismus von Medikamenten kann auch durch Bestandteile aus der Nahrung, Getränken oder Kräutern stark beeinflusst werden. Furanocoumarine aus der Grapefruit inhibieren beispielweise CYP 3A4 und 1A2 im Gastrointestinaltrakt (57,58). In der Transformer-Datenbank kann der Medikamentenmetabolismus von Phase I bis III nachvollzogen werden und mögliche Interaktionen werden hervorgehoben (59). Es ist außerdem möglich, die unterschiedlichen Wechselwirkungen in einem Netzwerk zu visualisieren. Abbildung 5 zeigt die Netzwerkvisualisierung der oben genannten Kombination von ASS mit Amoxicillin inklusive des alternativen Medikaments Ciprofloxacin. Beteiligte Enzyme sind in ihrer 3D-Struktur gezeigt. In dieser Darstellung kann man gut erkennen, dass Ciprofloxacin einen anderen Metabolismus durchläuft, als die beiden anderen Medikamente und es daher nicht zu (unerwünschten) Interaktionen kommen kann.

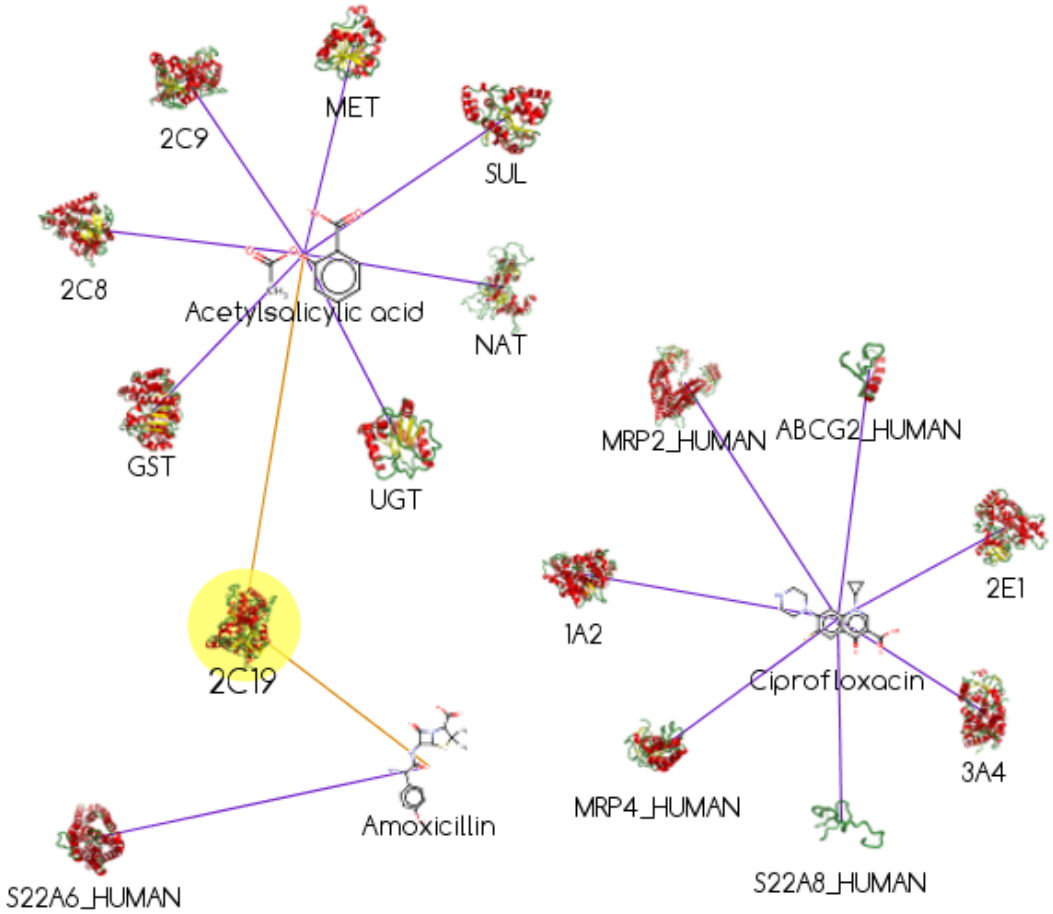

Abbildung 5: **Netzwerkvisualisierung dreier Medikamente.** In 2D-Struktur sind die Medikamente dargestellt, in 3D-Struktur die am Metabolismus beteiligten Enzyme. Die Interaktion zwischen Acetylsalicylsäure und Amoxicillin besteht durch CYP 2C19, während Ciprofloxacin über andere Enzyme verstoffwechselt wird.

Darüber hinaus werden Interaktionen von über 350 Nahrungsbestandteilen mit den diversen Enzymen angezeigt, sodass auch Patienten sich schnell und einfach informieren können, auf welche Nahrungsmittel sie unter Umständen verzichten sollten.

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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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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

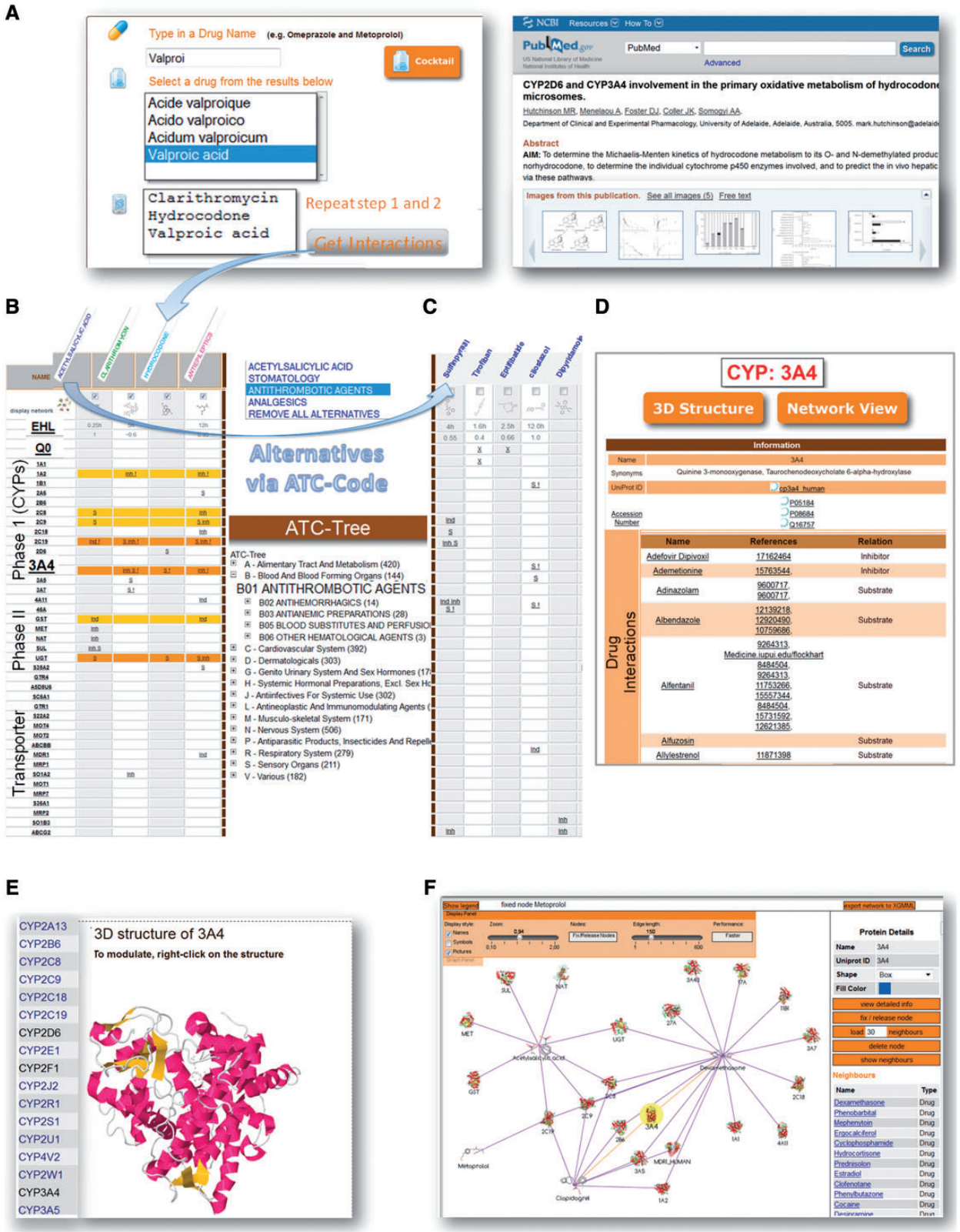

Figure 1. Functionalities of the 'Transformer database'. (**A**) Composition of a drug-cocktail. An example of a PubMed reference is shown. PubMed references can be viewed by clicking on 'S', 'Inh' or 'Ind' in the result table. (**B**) Clicking on 'Get Interactions' leads to a result table, which shows 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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2.5 Drug-Cocktail Optimierung

Mit der Verwendung des Wissens über Medikamentenmetabolismus, individuelle Polymorphismen und Medikamentenwechselwirkungen wäre ein großer Schritt in Richtung der personalisierten Medizin getan (60,61). Die Notwendigkeit zur Personalisierung der Medikamentengabe kann besonders eindrucksvoll am Beispiel von Therapie-Protokollen bei der Chemotherapie gezeigt werden, da Patienten eine umfangreiche Anzahl an antineoplastischen und Begleitmedikamenten einnehmen müssen, die sich gegenseitig beeinflussen und teilweise Prodrugs sind (62). Individuelle Polymorphismen der Patienten können schwere Nebenwirkungen und/oder Unwirksamkeit einzelner Medikamente hervorrufen, die bei der Chemotherapie mitunter die Prognose drastisch verschlechtern (63). Abbildung 6 zeigt das Ergebnis einer in Kooperation mit der pädiatrischen Onkologie der Charité durchgeführten retrospektiven Analyse von 143 Patienten mit einem späten Rezidiv von akuter lymphatischer Leukämie (ALL), die vom 1.1.2002 bis 31.12.2011 behandelt bzw. nachuntersucht wurden. Die inkludierten Patienten befanden sich in der intermediären Risikogruppe (S2) und wurden nach dem Protokoll ALL-REZ BMF 2002 II-IDA behandelt, das die folgenden Medikamente beinhaltet: Cytarabine, Vincristine, Mercaptopurin, Daunorubicin, Ifosfamid, Thioguanin, Dexamethason, Prednisolon. Knochenmarks- bzw. Blutproben wurden während der Therapie, sowie in Remissionsphasen genommen. Von den Remissionsproben wurden mononukleäre Zellen isoliert und DNA für die Genotypisierung extrahiert. Insgesamt überlebten 120 Kinder, während 23 während der Therapie oder aufgrund eines weiteren Rezidivs verstarben. Es konnte festgestellt werden, dass 18 SNPs zwei- bis sechsfach häufiger in der Gruppe der verstorbenen Patienten auftraten. Sieben der 18 SNPs traten ausschließlich in der Gruppe der verstorbenen Patienten auf und zehn der SNPs betrafen CYP 2A6, das für die Aktivierung von Ifosfamid und Cyclophosphamid verantwortlich ist. Hierdurch lassen sich unter anderem Toxizitäten und/oder Unwirksamkeit der Chemotherapeutika erklären. In einer

Replikationskohorte der Vanderbilt University konnten diese Ergebnisse validiert werden, wobei aufgrund der relativ geringen Fallzahl weitere klinische Studien notwendig werden, um ein Set an SNPs zu definieren, das vor Therapiebeginn bei allen Patienten untersucht wird. Durch die individuelle Genotypisierung und die entsprechende Anpassung der Medikamente in Dosis und/oder Stoffwechselweg, könnten Patienten effektiver und sicherer behandelt werden. Einer prospektive Studie in der auch Plasmakonzentrationen nach Medikamentengabe analysiert werden sollen, um Dosisanpassungen bei entsprechenden CYP-SNPs errechnen zu können, ist in Planung.

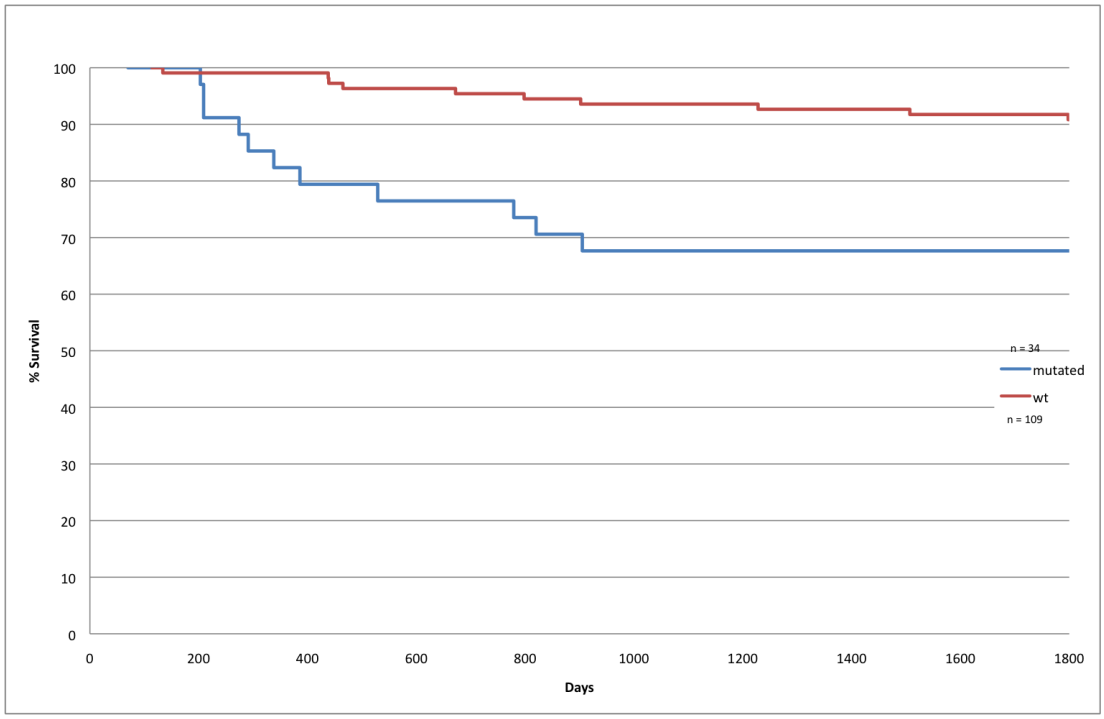

Abbildung 6: **Überlebenskurve von 143 pädiatrischen Patienten mit akuter lymphatischer Leukämie (ALL).** Für Patienten mit typischer Ausstattung an metabolischen Enzymen ist die Prognose deutlich besser (25% höhere Überlebensrate) als für diejenigen mit relevanten Mutationen in CYPs. Als Ursache scheint sich die fehlende Aktivierung von Fosfamiden abzuzeichnen.

Die Drug-Cocktail Optimierung spielt auch in der Schmerztherapie eine wichtige Rolle, da Schmerzmedikamente mitunter durch bestehende Medikation weniger bzw. teilweise unwirksam werden können.

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PLOS ONE

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:

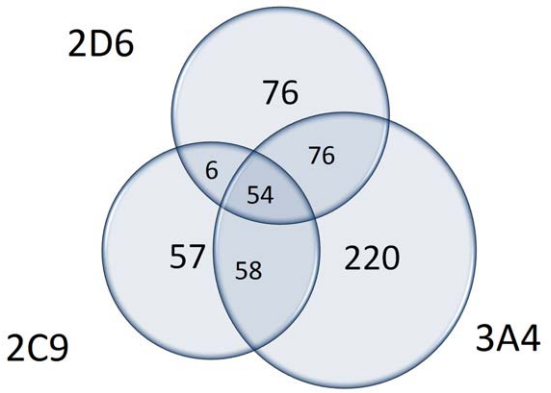

Figure 1. The Venn diagram illustrates the enzyme overload of CYPs 3A4, 2C9, 2D6 in chemotherapy. The numbers within the circles represent the drugs, which are metabolized by the CYPs. Intersection areas show the drugs, which are metabolized by two or three of the CYPs.
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1. 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-

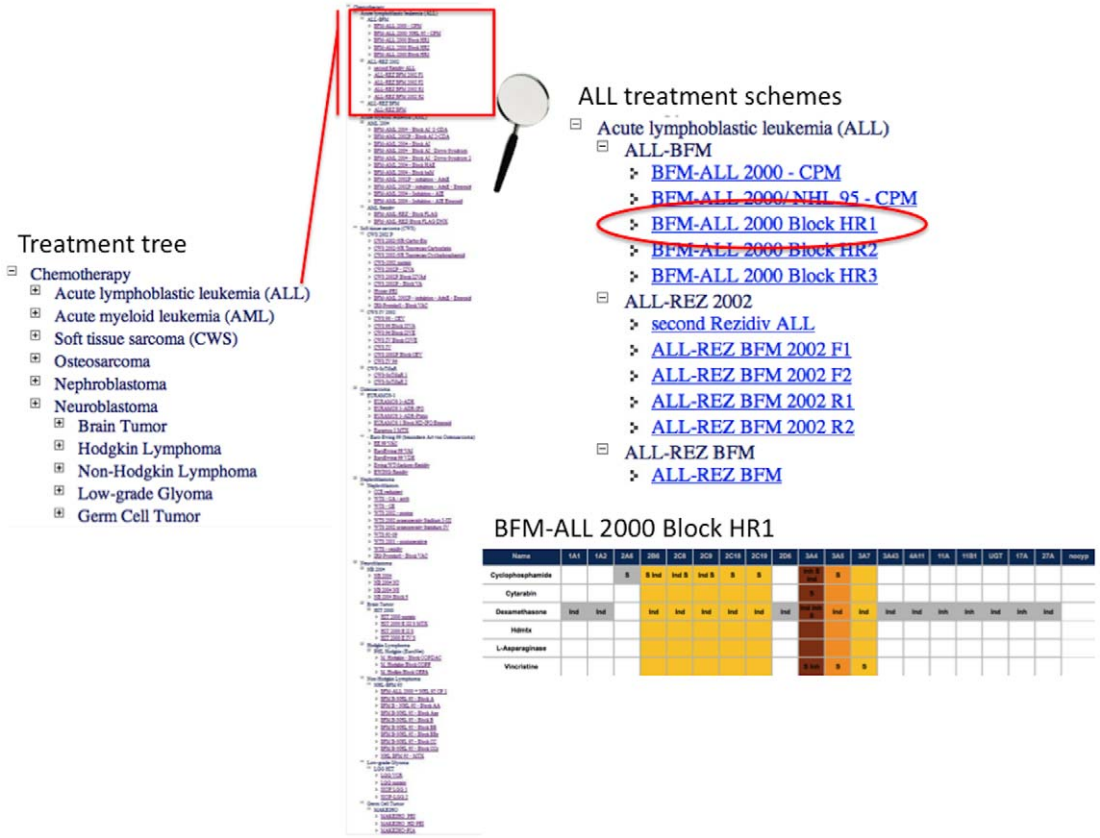

Figure 2. Treatment algorithm: Different antineoplastic treatment regimens in chemotherapy, ordered by diseases. By clicking on one of the diseases, different treatment options open up. After choosing one treatment regimen the metabolism of that drug-cocktail is illustrated.
doi:10.1371/journal.pone.0051020.g002

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.

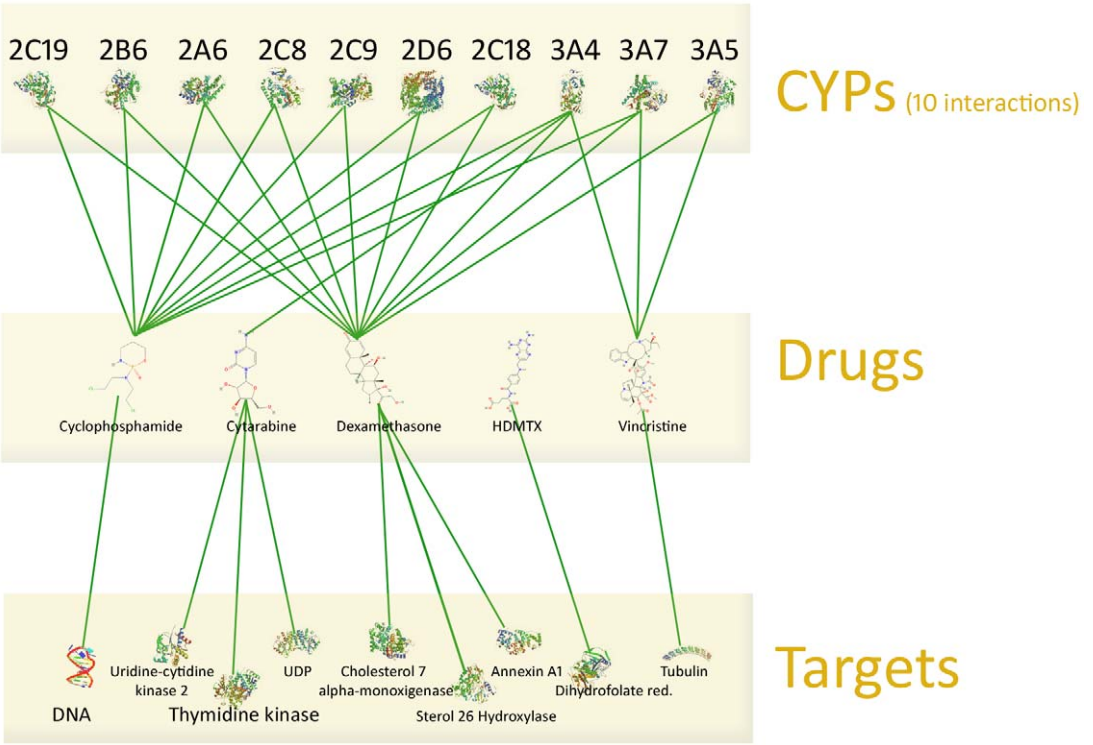

Figure 3. CYP interactions and targets of treatment regimen before optimization. The drugs of the medication are listed centrally in the Figure. Several green lines heading upwards illustrate ten CYPs, which are involved in the metabolism. The green lines heading downwards show the targets, which are 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.
doi:10.1371/journal.pone.0051020.t002

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

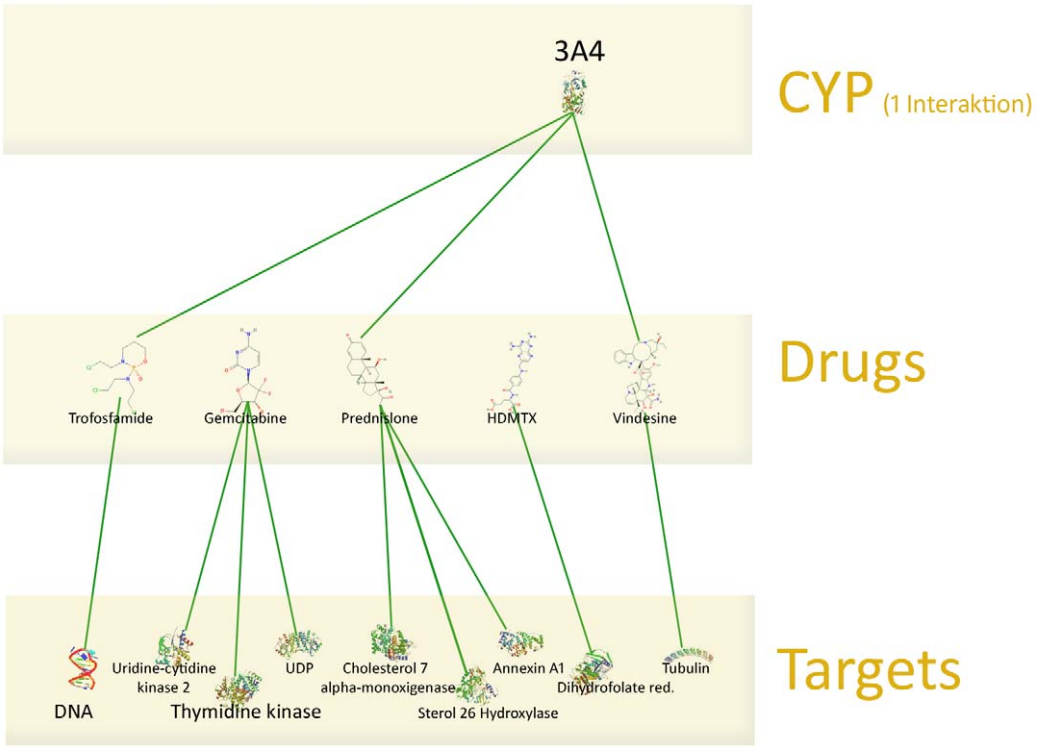

Figure 4. CYP interactions and targets of treatment regimen after optimization. By choosing drugs from the same ATC group with different metabolism pathways, only one CYP interaction remains.
doi:10.1371/journal.pone.0051020.g004

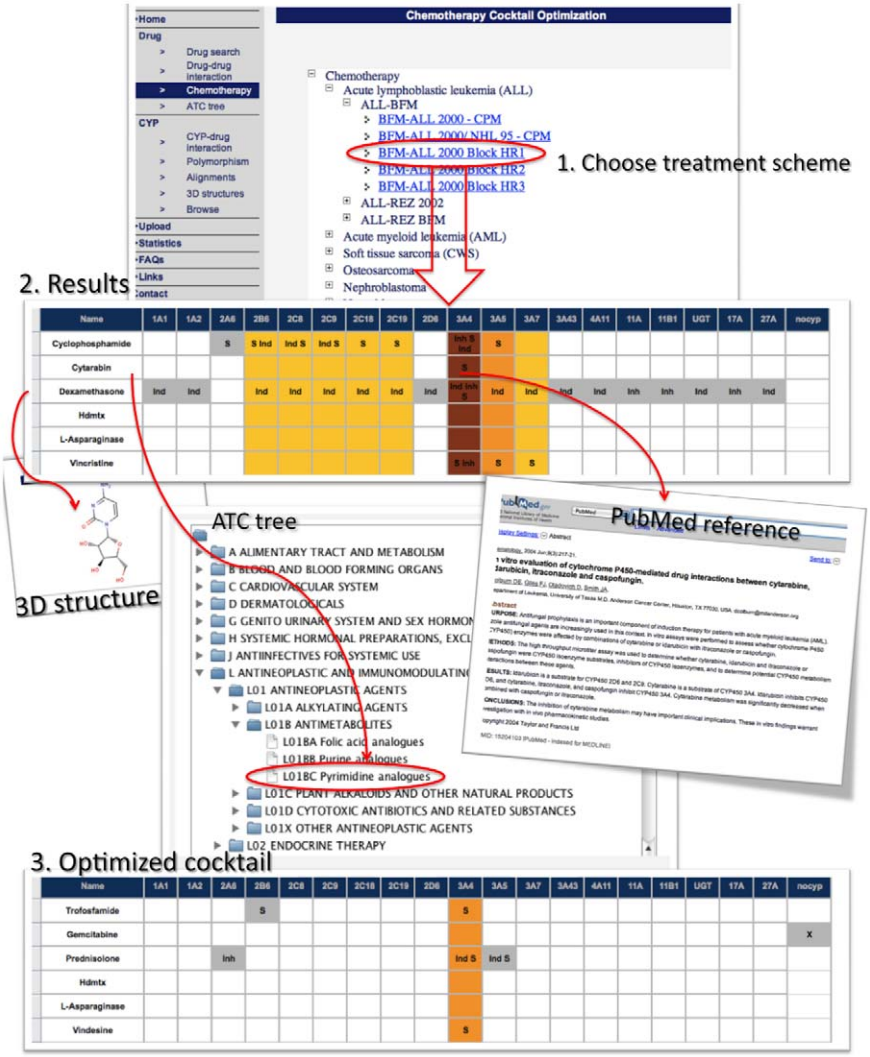

Figure 5. Optimization tool. Clicking on "Chemotherapy" in the navigation directs to the treatment tree, enabling to browse through different treatment regimens ordered by diseases. Once a treatment regimen is chosen, the drug-cocktail is shown on the "Results" page. The enzyme overload is visualized 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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3.Diskussion

3.1 Verwendung von Schmerzmitteln

Die meisten peripher wirksamen Schmerzmedikamente, wie ASS, Ibuprofen und Paracetamol können von Patienten bis zu einer bestimmten Dosierung rezeptfrei in jeder Apotheke bezogen werden. Insbesondere bei älteren Patienten mit bestehenden Komedikationen besteht eine große Wahrscheinlichkeit, dass die zusätzlich eingenommenen Schmerzmedikamente sich auf den Metabolismus der Standardmedikation auswirken (64). Dies kann sich einerseits durch geringere oder keine Wirkung der Schmerzmedikamente oder durch zu hohe/niedrige Spiegel der bestehenden Komedikation bemerkbar machen. Viele Patienten nehmen dann bei akuten Zahnschmerzen häufig deutlich mehr als die maximal empfohlene Dosis an Schmerzmedikamenten ein, bevor sie beispielsweise im zahnärztlichen Notdienst vorstellig werden (65,66). Mithilfe der entwickelten Datenbanken zur Überprüfung von Medikamentenwechselwirkungen können zunächst die Probleme bei der Verschreibung von Schmerzmitteln und/oder Antibiotika gelöst werden. Zum anderen wird aufgrund diverser neuer Erkenntnisse über Schmerzrezeptoren bei der Entwicklung von neuen Medikamenten zunehmend darauf geachtet, dass die richtigen Zielmoleküle erreicht werden. Erste Schritte in diese Richtung wurden bei nicht-steroidalen Antirheumatika mit löslicheren Präparaten verfolgt (Ibuprofen in Kombination mit Lysin, Natrium, Arginin) (22). Inzwischen sind die Kristallstrukturen von diversen Schmerz relevanten Rezeptoren aufgeklärt (41,67,68) und die Suche nach effektiveren Wirkstoffen mit gezielter Wirkung – und somit weniger Nebenwirkung – hat begonnen (32,35,37,69).

3.2 Polypharmazie

Die Anzahl an verschriebenen Medikamenten steigt seit vielen Jahren stetig an (70). In einer australischen Studie wurde festgestellt, dass 87,1% der über 50-Jährigen Australier mindestens ein Medikament pro Tag einnehmen und 43,3% mehr als fünf Medikamente pro Tag (71). Polypharmazie bezeichnet die Einnahme von mehr als fünf Medikamenten pro Tag, wodurch ein erhöhtes Risiko für unerwünschte Arzneimittelwirkungen entsteht. Die Häufigkeit von unerwünschten Nebenwirkungen korreliert mit der Anzahl der verschriebenen Medikamente (72). Schwere unerwünschte Arzneimittelwirkungen gehören bei stationären Patienten zu den häufigsten Todesursachen (73). Eine Ursache sind altersbedingte Veränderungen, die ältere Patienten anfälliger für unerwünschte Nebenwirkungen machen (74), aber die Tatsache, dass individuelle Polymorphismen den Medikamentenstoffwechsel signifikant verändern können, spielt ebenfalls eine wichtige Rolle (75).

3.3 Polymorphismen

Das Ziel einer jeden medikamentösen Behandlung sollte es in Zukunft sein, diese individuell auf den Patienten abzustimmen. Die Europäische Kommission widmet einen Großteil der Forschungsförderung im “Horizon 2020“-Programm der personalisierten Medizin, was zum einen den Bedarf deutlich macht, zum anderen aber auch zeigt, dass durch die Fortschritte der letzten 20 Jahre eine individuelle Therapie jetzt möglich wird. Besonders relevant sind genetische Tests vor allem für Patienten, die chemotherapeutisch behandelt werden, da die verabreichten Medikamente in hohen Dosen eingesetzt werden und malfunktionelle Enzyme dann lebensbedrohliche Toxizitäten verursachen können (76,77). Aber auch Patienten unter Therapie mit Statinen, Blutgerinnungshemmern oder Schmerzmedikamenten werden von flächendeckenden Genotypisierungen profitieren.

4.Zusammenfassung

Die Systembiologie bedient sich eines ganzheitlichen Ansatzes und integriert dabei ‚omics'-Daten verschiedener Ebenen in Computermodellen. Neue Ansätze sind darauf gerichtet, diese Methoden für medizinische Fragestellungen nutzbar zu machen. Zahnschmerz ist ein komplexes Geschehen, dessen Therapie die Integration individueller Daten erfordert. Eine Reihe von Rezeptoren – in der Regel Ionenkanäle – sind für die unmittelbare Schmerzvermittlung zuständig. Je nach betroffenem Gewebe sind andere Rezeptoren, deren 3D-Strukturen zunehmend bekannt sind, verantwortlich, was sich aus Expressionsanalysen ableiten lässt. Daraus lassen sich die jeweils geeigneten Therapeutika ableiten bzw. neue entwickeln. Häufig bekommen die Patienten allerdings weitere Medikamente, wodurch die Wirkung der Schmerzmedikamente beeinträchtigt sein kann bzw. unerwünschte Nebenwirkungen auftreten. Durch eine umfassende Analyse des Stoffwechsels des gesamten Medikamenten-Cocktails kann eine optimierte medikamentöse Therapie erreicht werden. Leider gehören die CYPs zu den besonders polymorphen Enzymen, so dass die Berücksichtigung verschiedener Allele mittlerweile bei der Gabe diverser Medikamente obligatorisch ist. Die Analysen des 1000-Genom-Projektes und weiterer Datenbanken zeigen, dass die Variabilität noch größer ist als bisher angenommen. Neben einem online verfügbaren Schema zum Medikamentenersatz bei CYP-Mutationen konnte im Rahmen dieser Arbeit durch eine Expressionsanalyse von 50 humanen Geweben aus jeweils 100 Patienten aufklären, in welchen Zielgeweben welches CYP stark exprimiert wird – ein Aspekt, der bisher kaum betrachtet wurde. Medikamente werden in drei Phasen des Stoffwechsels transformiert, wobei die CYPs nur die erste Phase katalysieren – es folgt die Konjugations- und die Transport-Phase. Nur die Gesamtbetrachtung – einschließlich Halbwertzeit, renaler Exkretion etc. – ermöglicht eine fundierte Drug-Cocktail-Optimierung. Die Praxis zeigte, dass darüber hinaus die Berücksichtigung bestimmter Nahrungsmittel von Bedeutung ist, was in das Computer-Modell integriert wurde. Die entwickelten Modelle sind online verfügbar, werden von 100 Nutzern täglich verwendet und finden aufgrund ihrer Allgemeingültigkeit nicht nur Verwendung in der Zahnmedizin.

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Erklärung

§4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde
- die vorliegende Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Datum Unterschrift