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## **Pediatric Pharmacogenomics: A systematic assessment of ontogeny and genetic variation to guide the design of statin studies in children**

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### **Synopsis**

The dose-exposure-response relationship for drugs may differ in pediatric patients compared to adults due to developmental changes in processes involved in drug disposition (absorption, distribution, metabolism and excretion) and drug response. This relative knowledge deficit has complicated drug efficacy and safety labeling of drugs for pediatric use. With the legislative changes that have occurred in the US and Europe over the last 20 years, many clinical studies have been conducted to establish drug dose-exposure relationships across the pediatric age spectrum from birth to adolescence. However, genetic variation has seldom been included in these investigations. This article applies a systematic approach to determine the relative contribution of development and genetic variation on drug disposition and response using HMG-CoA reductase inhibitors as a model. Application of the approach drives the collection of information relevant to understanding the potential contribution of ontogeny and genetic variation to statin dose-exposure-response in children, and identifies important knowledge deficits to be addressed through the design of future studies.

### **Keywords**

Pharmacogenomics; ontogeny; HMG-CoA reductase inhibitors; statins; low-density lipoprotein cholesterol; OATP1B1; cytochrome P450; pediatrics

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There has been extensive reform in pediatric drug labeling accomplished over the last twenty years as a direct result of new federal laws and regulation. In 1994, the US Food and Drug Administration (FDA) called for drug manufacturers to determine if existing data was sufficient for pediatric drug labeling<sup>1</sup>. Participation in this endeavor was subpar and therefore, the FDA Modernization Act (FDAMA) was enacted in November 1997. This legislation provided an additional six month patent exclusivity to manufacturers that conduct pediatric clinical trials according the FDA parameters<sup>2</sup>. A detailed review of the chronological events from 1994–2002 are provided by Steinbrook<sup>3</sup>. In January 2002, the Best Pharmaceuticals for Children Act (BPCA) provided further opportunities for drug manufacturers to generate data on drugs that were off-patent or patented drugs that has not

been studied in children<sup>4</sup>. One year later, the Pediatric Research Equity Act (PREA) enabled the FDA to require pediatric studies<sup>5</sup>. Overall, the results from this legislation have led to dramatic increase in pediatric studies on greater than 300 drugs and biological products. The plethora of new information has illuminated the continued challenge of appropriate pediatric drug dosing, efficacy, and safety.

As is widely appreciated in pediatric medicine, the changes that occur as children grow and develop influence the diagnosis and treatment of clinical disease. Merely extrapolating from adult therapeutic data may overlook the influence that developmental changes in expression of genes responsible for drug disposition have on dosing requirements and safety profiles of drugs that have distinct variation from birth until adulthood. Pharmacotherapy in children, like adults, is dependent upon clear understanding of the dose-exposure-response relationship of the drug to be administered. However, extrapolation of adult experience to pediatric age groups is complicated by age-associated differences in pharmacokinetics of several drugs used clinically in children<sup>6</sup>. In the past decade ontogeny of drug disposition, specifically in the domain of hepatic drug metabolizing enzymes, has been discovered<sup>7</sup>. However, our understanding of genetic variation's impact on drug disposition and efficacy in pediatrics still is lacking<sup>8,9</sup>. As expected though, the difficulty in performing prospective pediatric studies, due to ethical challenges and/or inadequate participation, have limited this greater understanding.

Understanding the relative contribution of ontogeny and genetic variation to observed variability in drug disposition and response in children challenges all parties involved in pediatrics drug research. The implementation of pharmacogenetic and pharmacogenomic strategies in children serves as another barrier to improve pediatric drug therapeutics. In the absence of more comprehensive data, a systematic approach has been developed to gather more information about certain drugs, identify knowledge gaps, and design studies to address those deficits. This approach has been used previously to address the dilemma of over the counter cough and cold preparations<sup>10</sup>. Our goal in this paper is to illustrate the use this systematic approach to assess current knowledge regarding the effects of ontogeny and genetic variation on the dose-exposure-response of a drug class whose use in pediatrics is anticipated to increase in the near future.

## Evolution of Statin Therapy in Children

Cardiovascular disease remains the number one cause of mortality in the United States despite significant progress in medical and invasive treatments<sup>11</sup>. Although symptoms typically appear in the 5<sup>th</sup> and 6<sup>th</sup> decades of life, atherosclerotic coronary artery disease (CAD) has its origins in childhood. In 1953, autopsies performed on 300 U.S. servicemen in their 20's revealed that over 75% had evidence of coronary atherosclerosis<sup>12</sup>. Another autopsy study of U.S. soldiers killed in the Vietnam War showed a 45% rate of atherosclerosis<sup>13</sup>. In a subsequent study involving young children and adolescents, fatty streaks, clinically silent precursors to CAD, were observed in the aortas of all children after the age of 3 years and progressed rapidly to coronary involvement by adolescence. Advancement to fibrous plaques mostly occurred in the third to fourth decades<sup>14</sup>. The Pathobiological Determinants of Atherosclerosis in Youth study and the Bogalusa Heart

Study noted varying stages of atherosclerosis in young children and youth with elevated low-density lipoprotein cholesterol (LDL) and other risk factors such as obesity, hypertension, tobacco smoke exposure and diabetes<sup>15, 16</sup>. These landmark studies have highlighted the need for implementing lipid screening and preventive cardiovascular measures during childhood.

The prevalence of total cholesterol (TC) >200mg/dl has risen to 10% in adolescents<sup>17</sup>. Epidemiological studies have documented that 75% of children with a TC concentration greater than the 90<sup>th</sup> percentile have TC concentrations >200mg/dl in their early twenties<sup>18</sup>. Elevated cholesterol is commonly associated with being overweight or obese. An alarming one-third of 2 to 19 year olds in the United States are diagnosed as overweight with a body mass index greater than the 85<sup>th</sup> percentile for age and sex<sup>19</sup>. A 55-year observational study showed that being overweight in adolescence resulted in a 2 fold higher relative risk of CAD mortality, independent of adult weight<sup>20</sup>. With the increasing prevalence of overweight children, the prevalence of clinically diagnosed CAD in young to middle age adults is expected to increase by 5–16% by the year 2035<sup>21</sup>.

In 1992, the National Cholesterol Education Program recommended lipid screening for children with a family history of premature CAD or dyslipidemia and in children with other risk factors such as obesity, hypertension, and diabetes mellitus<sup>22</sup>. This screening strategy has uncovered more cases of subclinical dyslipidemia that, without screening, would have been unrecognized for decades. More recent data have revealed that using family history alone to select children for lipid screening misses many patients with moderate acquired dyslipidemia and genetic dyslipidemia who may require pharmacologic treatment<sup>23</sup>. Therefore, updated guidelines from the American Academy of Pediatrics now recommend *universal* lipid screening at ages 9 to 11 years, and again at ages 18 to 21 years<sup>24</sup>.

Treatment strategies for dyslipidemia, including lifestyle modifications and pharmacologic therapy, have been well established in adults. In those who fail lifestyle modifications, pharmacologic therapy is commonly implemented. Guidelines for diet and pharmacologic treatment in children have also been established<sup>24, 25</sup>. There are several classes of medication available for treatment of dyslipidemia. 3-Hydroxy-3-methyl-glutaryl Coenzyme A (HMG-CoA) reductase inhibitors (statins) are now the mainstay of pharmacologic treatment of adult and pediatric dyslipidemia due to their demonstrated efficacy in the primary and secondary prevention of coronary artery disease and relatively mild side effect profile<sup>26–30</sup>. HMG-CoA reductase inhibitors decrease the hepatic synthesis of cholesterol by blocking the conversion of HMG-CoA to mevalonate, which is the rate-limiting step in cholesterol synthesis (Figure 1). The LDL-C receptor genes respond to this decrease of intracellular sterol by upregulating cell-surface LDL-C receptor expression<sup>31</sup>, which ultimately decreases the serum LDL. Furthermore, statins' pleiotropic effects include the decrease of inflammatory mediators downstream from HMG-CoA reductase. This pleiotropic effect could ultimately provide efficacy in other disorders of childhood inflammation beyond the scope of dyslipidemia. For example, patients with sickle cell disease can develop oxidative stress and chronic inflammation to their distal vasculature as a result of transient vaso-occlusion and subsequent reperfusion injury<sup>32</sup>. Hoppe *et al* found that biomarkers of vascular dysfunction, including C-reactive protein and interleukin 6, were

decreased in adolescents with sickle cell disease from 50% up to 70% after a 3 week trial of low (20 mg) or moderate (40mg) doses of simvastatin<sup>33</sup>. Additionally, statins have been utilized after cardiac transplantation to prevent coronary allograft vasculopathy (CAV). In pediatric cardiac transplantation, the prevalence of CAV is less pronounced compared to adults, but has been reported to be as high as 17% in one retrospective analysis<sup>34</sup>. LDL levels >100mg/dl, greater than optimal and near adult treatment range, have been reported in 39% of pediatric patients 1 year after transplantation<sup>35</sup>, which can be secondary to post-transplant steroid and cyclosporine therapy. Addition of pravastatin therapy in pediatric cardiac transplant recipients yielded a lower incidence of CAV<sup>34</sup>. Overall, statins are usually well tolerated and result in a 20–50% reduction in cholesterol from baseline<sup>36</sup>. Available information on statin use in pediatrics implies that statins are being used conservatively in children, estimated to be 1:4,500 children<sup>37</sup>. However, this crude estimate is likely to underestimate current use as it is derived from an analysis of Medicaid data from 2000 and a commercial Caremark database from 2004<sup>38</sup>, and preceded the increase in obesity and type II diabetes in children that has occurred over the past decade.

There are currently seven FDA approved statins - lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin, and pitavastatin. The majority of statin trials in pediatric subjects have involved lovastatin, simvastatin, and pravastatin<sup>28</sup>. Lovastatin, the first statin developed in the late 1980s, is a lipophilic, semi-synthetic inhibitor of HMG-CoA reductase. It is administered as an inactive lactone prodrug and is hydrolyzed in the liver to its active metabolites<sup>39</sup>. Simvastatin, introduced in the early 1990s, is also a lipophilic, semi-synthetic inhibitor of HMG-CoA reductase and administered as an inactive lactone prodrug that undergoes carboxylesterase-mediated conversion in the plasma, liver and intestine to simvastatin acid, which is the active metabolite<sup>40</sup>. Pravastatin, introduced in the early 1990s, is a hydrophilic, semi-synthetic inhibitor of HMG-CoA reductase<sup>41</sup>. Due to its hydrophilic nature, it fails to cross the blood brain barrier, making it a potentially safer alternative for maturing brains in children. Unlike other statins, it is not significantly metabolized by cytochrome P450 enzymes. In fact, the major metabolites are mainly produced in the acidic conditions of the stomach and are inactive<sup>42</sup>.

The majority of pediatric trials have focused on efficacy of lipid lowering and safety. The most recent double-blind, randomized, placebo-controlled, multicenter trial involving lovastatin (20 mg until week 4 then 40 mg from week 5 until week 24) in 54 postmenarchal females with familial hyperlipidemia between the ages of 10 to 17 years demonstrated a 23% reduction in LDL at 4 weeks and 27% after 24 weeks of treatment. Additionally, there were no clinically significant adverse effects observed between the two treatment groups over a 6 month period<sup>43</sup>. The largest double-blind, randomized, placebo-controlled multicenter trial with simvastatin (10 mg titrating up to 40 mg by week 24 continuing until week 48) in children ages 10 to 17 years by de Jongh et al demonstrated a 41% reduction in LDL, displaying simvastatin's efficacy in LDL reduction in children as well. There was a small decrease in dehydroepiandrosterone sulfate (DHEA) compared to subjects taking placebo, but no other changes in adrenal, gonadal, or pituitary hormones were observed in the treatment or placebo groups. No serious adverse drug events were reported in either treatment group<sup>44</sup>. Three previous double blind, randomized, placebo-controlled trials have demonstrated an approximate 25–35% reduction in LDL with pravastatin use in children,

validating its efficacy in this age group<sup>41, 45, 46</sup>. In addition to lowering LDL and total cholesterol, there is evidence that statin therapy in children with dyslipidemia can reverse increased carotid intima-medial thickness (IMT) and arterial endothelial dysfunction measured by ultrasound and flow-mediated dilation, respectively<sup>46, 47</sup>, which are biomarkers of the atherosclerotic process<sup>48–54</sup>. However, the studies presented above all involved a fixed dose of statin medication, and the effective dose received by each subject (mg per kg) would be expected to vary across the population, and the variability in dose administered alone could contribute to variability in response. For instance, de Jongh et al reported a mean decrease of 41% LDL cholesterol with a standard deviation of 39.2% at 48 weeks of simvastatin therapy<sup>44</sup>, and Wiegman et al reported that pravastatin was associated with a mean decrease in LDL of 24% with a range of 7–41%<sup>46</sup>. It is likely that additional factors, specifically ontogeny and genetic variation, will also contribute to variability in statin disposition and response in pediatric patients. These factors are discussed in more detail below.

## Contributions of Ontogeny and Genetic Variation in Drug Disposition

The relative lack of data regarding pediatric drug disposition is a limiting factor for optimal pediatric drug dosing strategies to maximize efficacy and minimize the potential for toxicity. Given that the use of statins can be anticipated to increase as a result of mandatory screening programs and difficulty with adherence to dietary and behavioral modifications, the pediatric community should be proactive in establishing therapeutic guidelines before statins are in widespread use. These therapeutic guidelines should be based on solid information concerning the dose-exposure-response relationship in pediatric patients, and studies designed to generate this information should take advantage of existing knowledge related to the contributions of ontogeny and genetic variation. The purpose of the remainder of this review is to present three fundamental issues that should be considered when assimilating current knowledge for application to problems related to variability in drug disposition and response in children. This systematic approach is applied to identify knowledge deficits related to the contribution of ontogeny and genetic variation to impact statin disposition and response in children, with implications for the design of future studies to address these knowledge deficits.

### Fundamental issues for assessing variability in drug disposition in children

**1. Knowledge of gene products that are quantitatively important in the disposition (absorption, distribution, metabolism, and excretion) of the drug(s) of interest?**—Simvastatin and lovastatin have been the two of the most commonly studied HMG-CoA reductase inhibitors in the pediatric population. They are both semi-synthetic, lipophilic compounds administered as a lactone prodrugs<sup>39, 55</sup> that are mainly absorbed from the gastrointestinal tract via passive diffusion and are subsequently hydrolyzed to active beta-hydroxy acid forms, simvastatin acid or lovastatin acid, in the liver<sup>39</sup>. *In vivo*, approximately 60–85% of the simvastatin prodrug is absorbed in the stomach, whereas only 30% of lovastatin prodrug is absorbed<sup>39, 56, 57</sup>. Due to their lipophilic nature, simvastatin and lovastatin are >95% protein bound in the plasma. Fluvastatin, atorvastatin, and pitavastatin are synthetic, lipophilic (although less than simvastatin and

lovastatin) compounds administered in their active form. They are absorbed rapidly via passive diffusion in the gastrointestinal tract secondary to their lipophilic nature and have a bioavailability of 30%, 12%, 51%, respectively. They are also highly protein bound due to their lipophilic nature<sup>39, 56, 58</sup>. Pravastatin remains a popular statin used in childhood and is labeled for use in children greater than 8 years of age. It is a hydrophilic, semi-synthetic compound that is administered in its active acidic form. Gastrointestinal absorption is estimated to be 30–35% due to its highly hydrophilic nature and reduced passive diffusion; absolute bioavailability is lower (17–18%) as a consequence of this incomplete absorption and first-pass metabolism. Due to its hydrophilic nature, it is only 50% protein bound<sup>39, 56</sup>. Rosuvastatin is a synthetic, hydrophilic (although less than pravastatin) compound that is administered in its active form. It also undergoes a slower absorption phase due to its less lipophilic nature, but protein bound is greater relative to pravastatin<sup>59, 60</sup>.

The liver is the major site of action and clearance for all statins used clinically. Hepatic uptake of statins is mediated by influx transporters known as organic anion transporting polypeptides (OATPs; Phase 0), followed by cytochrome P450 (CYP)-mediated oxidative metabolism for most statins (Phase 1), conjugation with glucuronic acid (Phase 2), and excretion of conjugated metabolites in the bile via the MRP family of efflux transporters (Phase 3)<sup>56, 61</sup>. These processes are summarized in Figure 2. Theoretically, any of these steps could be rate-limiting for statin clearance, but animal studies indicate that more comprehensive models that include hepatic uptake are superior to models based on metabolism alone in predicting *in vivo* statin clearance from *in vitro* systems<sup>62</sup>. Each of these four steps of statin disposition in liver will be discussed in more detail below. Emphasis will be paid to those processes that are *quantitatively* important in hepatic statin disposition to distinguish those processes that profoundly affect systemic statin exposure in humans from those that are merely *capable* of transporting or metabolizing statins based on data from isolated *in vitro* systems.

**Hepatic uptake:** Although statins may gain entry to hepatocytes by passive diffusion, the process is facilitated by a transporter-mediated system. The primary transporter mediating the hepatocellular uptake of statins is OATP1B1, the protein product of the *SLCO1B1* gene, and has been the subject of several comprehensive reviews<sup>56, 61, 63</sup>. For pitavastatin, OATP1B3 (*SLCO1B3* gene product) has been reported to play a minor role, but uptake primarily occurs by OATP1B1-mediated transport<sup>61, 64</sup>. Additionally, fluvastatin and rosuvastatin have been shown to be substrates of OATP1B3 and OATP2B1 mediated transport<sup>63, 65, 66</sup>. Although simvastatin and lovastatin can inhibit OATP1B1-mediated transport<sup>67</sup>, OATP1B1 appears less important for cellular uptake of these agents relative to other statins due to their highly lipophilic nature and greater role for passive diffusion<sup>68</sup>.

Inhibitors of OATP1B1 are of great utility to gain insight into the functional importance of OATP1B1 mediated statin uptake. For example, concurrent administration of a potent inhibitor of OATP1B1 would be expected to increase the systemic exposure (as determined by an increase in total area under the curve, or AUC) for those statins that rely on OATP1B1 for hepatic drug uptake. Theoretically, the greater the increase in AUC in the presence of inhibitor, the greater the role of OATP1B1 in mediating hepatic drug uptake as reduced entry into the liver is accompanied by an increase in the statin concentration circulating in plasma;

if a particular statin is not dependent on OATP1B1 for cellular uptake, the AUC will not be affected by the presence of an inhibitor. The quantitative importance of OATP1B1 for statin uptake *in vivo* has been established using rifampin, a known inhibitor of OATP1B1 and OATP1B3 *in vitro*. For example, concurrent administration of a single 600 mg dose of rifampin resulted in a 6-fold increase in the AUC of atorvastatin acid compared to atorvastatin alone<sup>69</sup>. Cyclosporine, a potent inhibitor of OATP1B1 and CYP3A4, increased atorvastatin AUC 7- to 15-fold, fluvastatin AUC 3- to 4-fold, lovastatin AUC 20-fold, pravastatin AUC 5- to 10-fold, pitavastatin AUC 5-fold, rosuvastatin AUC 7-fold, and simvastatin AUC 3- to 8-fold (reviewed in<sup>63</sup>). The potential contribution of CYP3A4 inhibition to the increased AUC of statins associated with cyclosporine is considered to be minor at best given that rosuvastatin, pravastatin, and pitavastatin are excreted unchanged and are not significantly metabolized by CYP3A4<sup>70</sup>. Pravastatin, a very hydrophilic compound that does not have significant passive diffusion capabilities, had a 10-fold increase in AUC when given in pediatric patient on immunosuppressive therapy containing cyclosporine compared to patients receiving pravastatin for familial hypercholesterolemia<sup>71</sup>, documenting the importance of OATP1B1-mediated transport of pravastatin into the hepatocyte. The effect of cyclosporine on rosuvastatin AUC (7-fold increase), another hydrophilic compound, is consistent with an important role for OATP1B1 in hepatic uptake<sup>72</sup>. Cumulatively, the data from studies with inhibitor studies provide convincing evidence that the OATP1B1 transporter is a critically important determinant of drug disposition for most of the statins. This quantitative importance of *SLCO1B1* is also confirmed by pharmacogenetics studies to be described below.

**Phase 1 metabolism:** Current evidence indicates that cytochrome P450 3A4 (CYP3A4) is the primary pathway for statin metabolism. Exceptions include pravastatin, pitavastatin and rosuvastatin, which do not undergo significant CYP-mediated metabolism<sup>58, 61, 64</sup>, and fluvastatin, which is a substrate for CYP2C9, based on both *in vitro* and *in vivo* data<sup>73-75</sup>. Although *in vitro* reaction phenotyping studies suggest that CYPs 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5 are capable of metabolizing various statins, CYP3A4 appears to be primarily responsible for the metabolism of simvastatin, lovastatin, and atorvastatin<sup>76-78</sup>, with CYP2C8 contributing to the metabolism of some statins. The quantitative importance of CYP3A4-dependent metabolism is illustrated by a 90% decrease in simvastatin acid metabolism in the presence of CYP3A4/5 inhibitor troleandomycin *in vitro*<sup>78</sup>, and by *in vivo* pharmacokinetic studies in which concurrent administration of CYP3A4 inhibitors such as itraconazole results in 15- to 19-fold increases simvastatin and lovastatin AUC<sup>56, 79-81</sup>. A more modest 47% increase in atorvastatin AUC is observed with co-administered itraconazole<sup>82</sup>. Administration of CYP3A4 inhibitors has no significant effect on clearance of pravastatin, fluvastatin, rosuvastatin, or pitavastatin, consistent with the limited role of CYP3A4 in the metabolism of these compounds<sup>70</sup>.

**Phase 2 metabolism:** Conjugation by conjugation with glucuronic acid catalyzed by UDP glucuronosyl transferases (UGTs) is the primary route by which statins and statin metabolites are further metabolized in hepatocytes<sup>83, 84</sup>. The open acid forms of the statins are glucuronidated by UGT to form an acyl glucuronide that subsequently cyclizes to form a lactone ring. This process of lactonization is a common metabolic pathway for all statins in



the open acid form, and results in a loss of pharmacologic activity. Statin lactones can be converted back to the open acid forms by carboxyl esterase and subject to further metabolism or excretion into the urine or bile; lactones can also be directly metabolized by cytochrome P450<sup>61</sup>. Fujino et al demonstrated that the statin lactones are more rapidly metabolized by cytochrome P450 than the statin open acids<sup>85</sup>. Concurrent administration of gemfibrozil, a fibrate that inhibits cytochrome P450 and UGT-mediated metabolism of simvastatin and atorvastatin, has been reported to increase the AUC of simvastatin acid, but not the lactone form, consistent with an inhibitory effect on the lactonization of simvastatin acid *in vivo*<sup>86</sup>. Overall, the contribution of UGT-dependent metabolism is considered to be substantially less than the role of CYPs<sup>85</sup>. Other statins, such as pravastatin, rosuvastatin, and pitavastatin, undergo excretion in their intact form and do not undergo extensive cytochrome P450 or UGT-mediated metabolism.

**Phase 3 cellular efflux:** Efflux of the conjugated statin metabolites occurs via several efflux transporters located on the canalicular membrane of the hepatocyte (Figure 2). The biliary excretion of statins is mediated by multiple transporters, including multidrug resistance associated protein 2 (MRP2; *ABCC2*), multidrug resistance 1 (MDR1; *ABCB1*), breast cancer resistance protein (BCRP; *ABCG2*), and bile salt exporting pump (BSEP; *ABCB11*). There is insufficient information to determine the quantitative importance of efflux transporters as determinants of the systemic exposure to statins.

When all phases of hepatocellular uptake and metabolism are considered, OATP1B1 appears to be a crucial determinant of statin drug disposition. Furthermore, CYP3A4 activity, and to a lesser extent CYP2C8, contribute to the disposition of statins that are substrates for CYP-mediated metabolism (*e.g.*, simvastatin, lovastatin, and atorvastatin). However, when a clinical cassette microdosing study design was employed to investigate the relative contribution of OATP1B1 and CYP3A4 toward atorvastatin disposition, AUC was increased 12-fold following inhibition of OATP1B1 by rifampin, but was unaffected by inhibition of CYP3A4 by itraconazole<sup>87</sup>. Thus, hepatic uptake by OATP1B1 appears to be the rate-limiting step in atorvastatin hepatic clearance.

**2. Identification of allelic variation in the genes of interest that are associated with functional consequences *in vivo*?**—The solute carrier organic anion transporter (*SLCO*) gene family codes for OATP transporters<sup>88</sup>, and the effect of genetic variation on statin disposition has been the subject of considerable interest<sup>63</sup>. *SLCO1B1* is expressed exclusively in the liver and its major role is drug and xenobiotic transport into the hepatocyte. The observation of extreme "high outliers" (n=4 of 84 healthy male volunteers) in a pharmacokinetic study of pravastatin<sup>89</sup> was subsequently attributed to two single nucleotide variants (SNVs) in *SLCO1B1*, -11187G>A in the promoter region and c.521T>C in exon 5, which were associated with a 50% reduction of non-renal clearance<sup>90</sup>. This effect was independently confirmed by haplotype analysis, in which heterozygous carriers of *SLCO1B1*\*15B (containing the 388A>G and 521T>C variants) had a mean pravastatin AUC 0–12 hours that was 93% higher compared to non-carriers, and heterozygous carriers of the \*17 haplotype (containing the -11187G>A, 388A>G and 521T>C variants) had 130% higher AUC compared to non-carriers<sup>91</sup>. Multiple *SLCO1B1* haplotypes have now been

described and haplotype frequencies vary across geographical regions. The combined frequency of low activity *SLCO1B1*\*5 and \*15 haplotypes is 15–20% in Europeans, 10–15% in Asians, and approximately 2% in sub-Saharan Africans, whereas the \*1B haplotype, which is generally considered to be associated with higher activity, ranges in frequency from 26% in Europeans to up to 77% in sub-Saharan Africans<sup>63</sup>. The functional consequence of *SLCO1B1* haplotype on statin AUC generally follows the dependence of individual statins on OATP1B1 for cellular uptake. Heterozygosity for *SLCO1B1*\*5 and \*15 haplotypes is associated with an approximately 3-fold increase in AUC for simvastatin acid, and 2.5-fold and 2-fold increases for atorvastatin and pravastatin, respectively; fluvastatin AUC appears to be least affected by *SLCO1B1* genotype<sup>63</sup>. The effect of rifampin on atorvastatin AUC is also dependent upon *SLCO1B1* genotype with a 9-fold increase in AUC associated with the fully functional *SLCO1B1* 521CC genotype compared to a 4-fold increase in AUC in subjects homozygous for the 521TT genotype associated with reduced transporter expression<sup>92</sup>. Thus, pharmacogenetic studies support a critical role for OATP1B1/*SLCO1B1* in statin disposition.

Allelic variation in *SLCO1B1* has important implications for drug safety as the increased systemic exposure associated with reduced activity haplotypes has the potential to increase the risk of myopathy in statin-treated patients. This relationship has been demonstrated by the STRENGTH (Statin Response Examined by Genetic Haplotype Markers) trial in which heterozygosity for a non-coding SNV in linkage disequilibrium with c.521T>C SNV was associated with a 4.5-fold increase in risk of myopathy, and increase to 16.9 in subjects homozygous for the SNV<sup>93</sup>.

The relationship between genetic variation in phase 1 metabolism and statin disposition is limited relative to *SLCO1B1* pharmacogenetics and cellular uptake. Although CYP3A4 activity is highly variable in humans, genetic determinants of the observed variability remain unclear<sup>94</sup>. Recently, Wang et al identified an SNV in intron 6 of *CYP3A4* (rs35599367 C>T) that has now been designated the *CYP3A4*\*22 allele. This variant was associated with 1.7- and 2.5-fold decreases in CYP3A4 expression and activity in heterozygous and homozygous carriers, respectively. In patients receiving stable doses of either atorvastatin, simvastatin, and lovastatin, individuals with a *CYP3A4*\*22 allele required a 0.2–0.6-fold lower dose of statin therapy for lipid control<sup>95</sup>, consistent with decreased CYP3A4 activity and reduced statin clearance. This effect has been replicated by Elens et al who studied 80 patients treated with simvastatin and observed that patients either homozygous or heterozygous for *CYP3A4*\*22 had a 0.25mmol/l and 0.29mmol/l reduction in total and LDL cholesterol, respectively, compared to those with homozygous wild type<sup>96</sup>. Thus, allelic variation in *CYP3A4* also appears to influence the pharmacodynamic impact of statins that are dependent upon this CYP for their metabolism. The *CYP2C9*\*3 allele has a much more dramatic effect on CYP2C9 activity, and patients homozygous for the \*3 allele had 3-fold lower clearance of the active fluvastatin enantiomer, but reduction in serum cholesterol was not related to *CYP2C9* genotype<sup>97</sup>.

Although phase 2 metabolism has a more limited impact on statin disposition compared to cellular uptake or phase 1 metabolism, recent work suggests that UGT allelic variants may have a modest effect of statin activity. Lactonization of atorvastatin has been attributed to

UGT1A3, and the *UGT1A3\*2* allele has been associated with increased lactonization activity<sup>98</sup>. The lactone has reduced clinical effect, and a study conducted in 23 healthy volunteers demonstrated that homozygosity of the *UGT1A3\*2* allele was accompanied by a 1.7- and 2.7-fold increase in AUC of atorvastatin lactone and 2-hydroxyatorvastatin lactone, respectively, compared to those homozygous for *UGT1A3\*1* allele. Furthermore, increase lactone formation correlated with a decreased effect on total and LDL cholesterol lowering from baseline<sup>99</sup>.

The functional consequence of genetic variation in phase 3 efflux transporters is limited relative to the role of cellular uptake. Studies of allelic variation in *ABCC2* reveal a dependence on *SLCO1B1* genotype<sup>100</sup>. Allelic variation in *ABCB1* does not appear to have any significant role in the interindividual variability in the pharmacokinetics of fluvastatin, pravastatin, lovastatin, and rosuvastatin<sup>101</sup>. The *ABCG2* c.421C>A variant has been associated with reduced transport activity in vitro<sup>102</sup>, and the AUC of atorvastatin, fluvastatin, simvastatin lactone, and rosuvastatin is reported to be 72%, 72%, 111% and 144% greater in subjects with a c.421AA genotype compared to wild-type c.421CC genotype group<sup>103, 104</sup>, but no significant impact on simvastatin acid or pravastatin pharmacokinetics<sup>103</sup>.

**3. Knowledge of the developmental profile (ontogeny) of key pathways involved in drug disposition?**—As presented above, *SLCO1B1* and *CYP3A4* have emerged as the primary determinants of statin disposition based on studies conducted in adults. Relative to drug metabolism, considerably less is known about the ontogeny of transporters (influx and efflux) during human development. Nevertheless, knowledge of ontogeny is essential for proper application and interpretation of pharmacogenetic data as genotype-phenotype relationships are only apparent once the gene is expressed, and are most stable when the gene is fully expressed. A comprehensive analysis of transporter mRNA expression in mice of different ages and developmental stages using next generation mRNA sequencing analysis revealed that the expression of transporters in liver is both age- and isoform-specific<sup>105</sup>. Of the 15 *SLCO* genes in mice, only five were expressed in liver, with two (*Slco1a4* and *Slco1b2*) being included in an adolescent-enriched group of transcripts, and three (*Slco1a1*, *Slco2a1* and *Slco2b1*) have adult-enriched patterns of expression. *Slco1b2* is considered to be the mouse homolog of human *SLCO1B1* and *SLCO1B3*, and showed a biphasic developmental profile with expression increasing rapidly after birth, peaking during adolescence (10–20 days postnatal age) and declining during the transition from adolescence to adulthood before eventually returning to adolescent levels of expression. The ontogeny of *SLCO1B1* in humans is not known, but if its ontogeny is as complex as mouse *Oatp1b2*, the functional consequence of *SLCO1B1* genetic variation in children may be difficult to across the developmental spectrum. Indeed, only one small pharmacokinetic/pharmacogenetic trial in children has been published to date. In 21 children with familial hyperlipidemia who received pravastatin, the *SLCO1B1* –1187GA genotype appeared to have the opposite effect from that observed in adults. Children with the variant SNV had an 81% lower peak pravastatin concentration (C<sub>max</sub>) and 74% lower AUC compared to children with the wild type (–1187GG) genotype in marked contrast to published adult experience in which the variant genotype was associated with higher AUC

values. Additionally, patients with the c.521T>C genotype had a 49% lower peak plasma pravastatin concentration and 26% lower AUC, but these differences did not achieve statistical significance<sup>106</sup>. This study suffers from a small number of children with the variant genotype, and genotype-phenotype relationships could also be confounded by concurrent administration of cyclosporine in the cardiac transplant patients included in the study. However, the changes in C<sub>max</sub> and AUC are opposite to what would be expected if cyclosporine was inhibiting residual transporter function in patients with the variant genotypes. Clearly, these preliminary findings need to be replicated in a larger group of patients, and the potential effect of age (ontogen) taken into consideration.

The ontogeny of CYPs and UGTs in humans appears to occur in distinct patterns<sup>7</sup>. *CYP3A4* is a member of a gene locus that contains three other members, *CYP3A5*, *CYP3A7* and *CYP3A43*. The ontogeny of *CYP3A7* is characteristic of the Group 1 pattern of expression proposed by Hines – high expression in fetal liver followed by decreasing expression after birth, and minimal expression in adults. *CYP3A5* protein and activity can be detected in fetal and postnatal liver, and genetic variation is a more important determinant of variability in expression than ontogeny. The developmental trajectory of *CYP3A4* follows the Group 3 pattern of expression in which functional *CYP3A4* activity is minimal in fetal liver, but increases after birth. *In vitro* studies conducted with a large panel of postmortem pediatric liver tissues indicates that *CYP3A7* activity in the first week of postnatal life is comparable to that observed in fetal liver, and declines by an order of magnitude over the first year of life. In contrast, *CYP3A4* activity is low at birth, demonstrates modest increases in activity over the first month, but remains less than that observed in adult level between 1 and 10 years of age<sup>107</sup>. These *in vitro* data imply that *CYP3A7* may be the dominant *CYP3A* isoform in the first year of life, with *CYP3A4* assuming increasing importance thereafter. *In vivo* data are consistent with acquisition of functional *CYP3A4* activity after birth and through the first year of life. Pharmacokinetic studies with midazolam, which is considered to be a prototypic *CYP3A4* substrate, and cisapride in neonates, consistently indicate that clearance increases with postnatal age<sup>108</sup>. Similarly, an investigation of sildenafil pharmacokinetics in newborns revealed that a 3-fold increase in drug clearance over the first week of life was accompanied by an increase in the formation of the *CYP3A4*-dependent *N*-desmethyl metabolite<sup>109</sup>. A longitudinal phenotyping study conducted in infants 2 weeks to 12 months of age also supported maturation of *CYP3A4* through an increase in *N*-demethylated metabolites of the cough suppressant dextromethorphan<sup>110</sup>. Estimates of weight-adjusted drug clearance (ml/min/kg) for *CYP3A4* substrates generally are higher in younger children necessitating higher weight-adjusted (mg/kg) doses than adults to achieve similar target concentrations<sup>108</sup>. However, these differences tend to be less pronounced when clearance (and dose) are adjusted for body surface area. For example, allometric scaling of sildenafil clearance indicates that adult levels are achieved by the end of the first week of life<sup>109</sup>. Complicating a clearer understanding of the ontogeny of drug metabolism is the fact that liver mass as a percentage of total body mass changes throughout childhood, being higher (3.5%, range 2.1% to 4.7%) in children 2 years of age compared to 2.2% (range 1.8% to 2.8%) in individuals over 18 years of age<sup>111</sup>. The issue of ontogeny is further confounded by possibility that the pattern of metabolites formed by children may differ from that observed in adults, as has been reported recently for sirolimus, a substrate of *CYP3A4* and

CYP3A5<sup>112</sup>. To our knowledge, the ontogeny of statin metabolism has not been investigated to date.

CYP2C9 ontogeny is relevant to fluvastatin metabolism and also demonstrates a Group 3 developmental profile. Similar to CYP3A4, estimates of weight-adjusted drug clearance and dose requirement are higher in young children than adults, but these differences largely disappear when developmental differences in organ size are taken into consideration<sup>108</sup>.

## Summary and Conclusions

Based on the American Academy of Pediatrics guidelines, approximately 0.8% of adolescents 12–17 years with dyslipidemia may qualify for pharmacological treatment. This translates into approximately 200,000 12–17 year olds eligible for statin therapy<sup>113</sup>. Given the ongoing childhood obesity epidemic, and the increased incidence of dyslipidemia associated with obesity, it is anticipated that the number of children and adolescents identified with dyslipidemia will continue to increase and some of these may ultimately require statin therapy. With the potential for increased use of statins in children and adolescents, it is imperative that we have improved understanding of the developmental characteristics affecting the pharmacokinetics and pharmacodynamics of statins in these pediatric populations. Simply extrapolating pediatric dosing guidelines from adult dose-exposure-response relationships fails to recognize the complexity of growth and developmental changes in pediatric patients, and the clinical implications for drug efficacy or adverse drug effects<sup>6</sup>. Interestingly, a recent study demonstrated that genetic risk scores derived from 95 SNVs associated with blood lipids in adults explained twice as much of the total variance in HDL-cholesterol, LDL-cholesterol and total cholesterol in 3- to 6-year old children compared to adults<sup>114</sup>. On the one hand, it is encouraging that genetic markers of risk derived from adult data are also applicable to children, but the data also imply that additional factors influence lipid levels in children and adults, and environmental factors cannot be ignored.

From the perspective of statin treatment, as summarized above available data from adult studies implicate hepatocellular uptake via OATP1B1 and CYP3A4-dependent metabolism as critical determinants of statin disposition. This analysis also identified important knowledge deficits relevant for pediatric investigations. First, the ontogeny of *SLCO1B1* in humans is unknown, and therefore it is not possible to predict the influence that developmental differences in OATP1B1 expression may have on statin systemic exposure at different ages/developmental stages. Second, without this information it is difficult to predict the effect of allelic variation in *SLCO1B1* on statin system exposure in pediatric populations as illustrated by the limited pediatric data to date<sup>106</sup>, nor when genotype-phenotype relationships observed in adults will become apparent in children. It is interesting to note in this regard that genotype-phenotype relationships for *ABCB1* were not apparent in children <8 years of age, but were observed in children 8 years of age and older<sup>115</sup>. Thus, genotype-aided pharmacokinetic studies are warranted in children and adolescents to resolve this matter and determine in age-related differences in the dose-exposure relationship are present. Finally, modeling studies suggest that OATP1B1 activity is the primary determinant of plasma statin concentration whereas intracellular statin concentrations are determined by

CYP and efflux transporter activity<sup>62</sup>. Thus, one cannot ignore the potential for developmental or pharmacogenetic differences in CYP3A4 activity to influence the inhibitory effects of statins on cholesterol biosynthesis.

The traditional model of clinical drug development is to investigate the effect of statins in populations, and then attempt to apply the data to treat individual patients. The problem is further complicated when the population experience is in adults, and the information is to be applied to pediatric patients of different ages. Therefore, there is a need to conduct studies to identify and quantify sources of inter-individual variability in statin disposition and response for the management of dyslipidemias in children and adolescents. The challenge for the future is address each of the knowledge deficits identified above to better characterize the dose-exposure-response relationship in children and adolescents such that the design of future clinical trials will be better informed, increasing the likelihood of clinically useful data and avoiding the mistakes of the past<sup>116</sup>.

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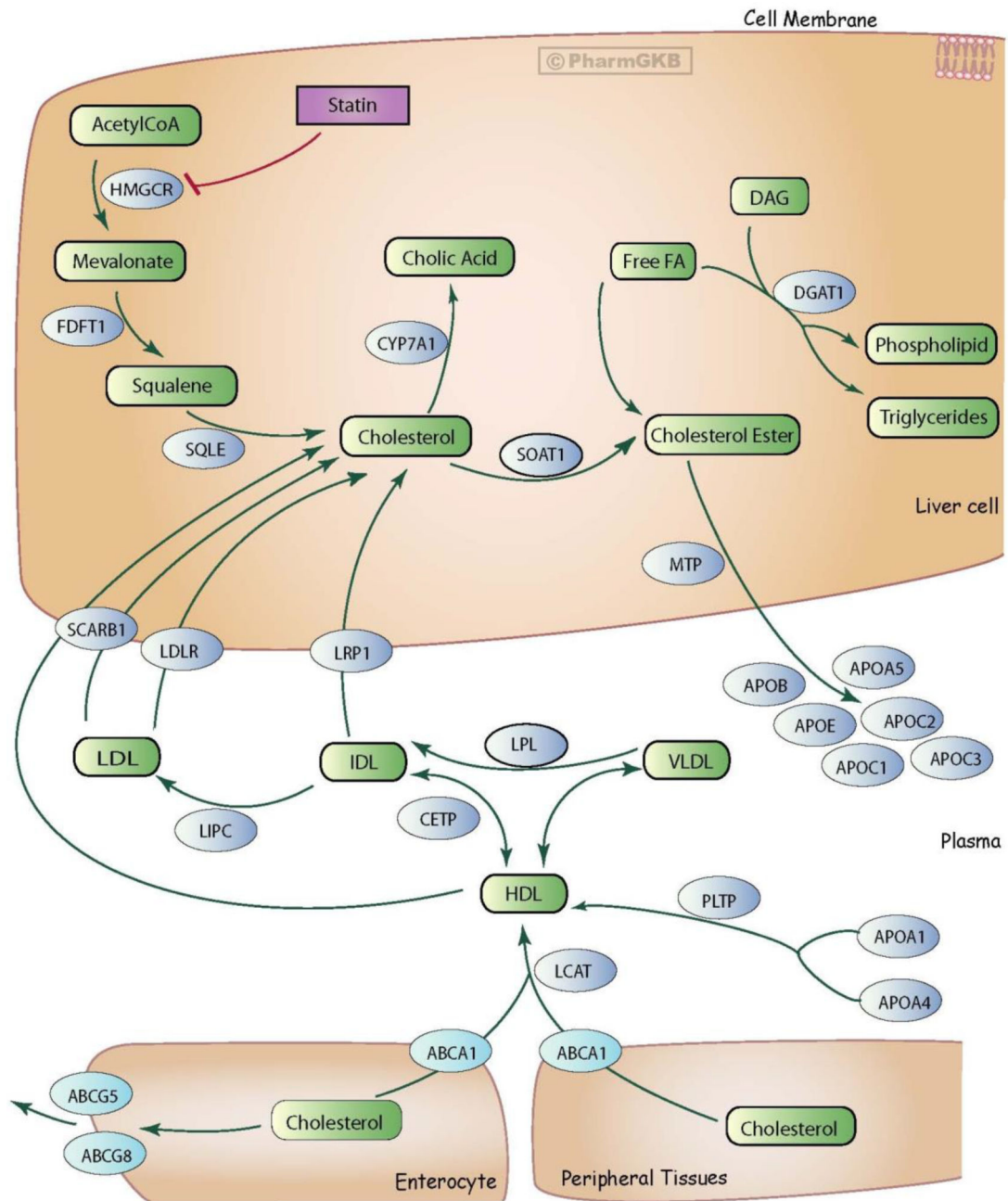
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**Figure 1. Cholesterol Biosynthesis Pathway**

Statins as a class inhibit endogenous cholesterol production by competitive inhibition of HMG-CoA reductase (HMGCR), which catalyzes conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol synthesis. The effect of statins is shown in the context of genes involved in the metabolism and transport of plasma lipoproteins that affect atherosclerosis and cardiovascular disease risk. A more detailed description of individual genes and gene products can be found at <http://www.pharmgkb.org/pathway/PA2031> (accessed May 14, 2012). The figure is copyrighted by the Pharmacogenomics Knowledgebase (PharmGKB; E.M. McDonagh, M. Whirl-Carrillo, Y. Garten, R.B. Altman

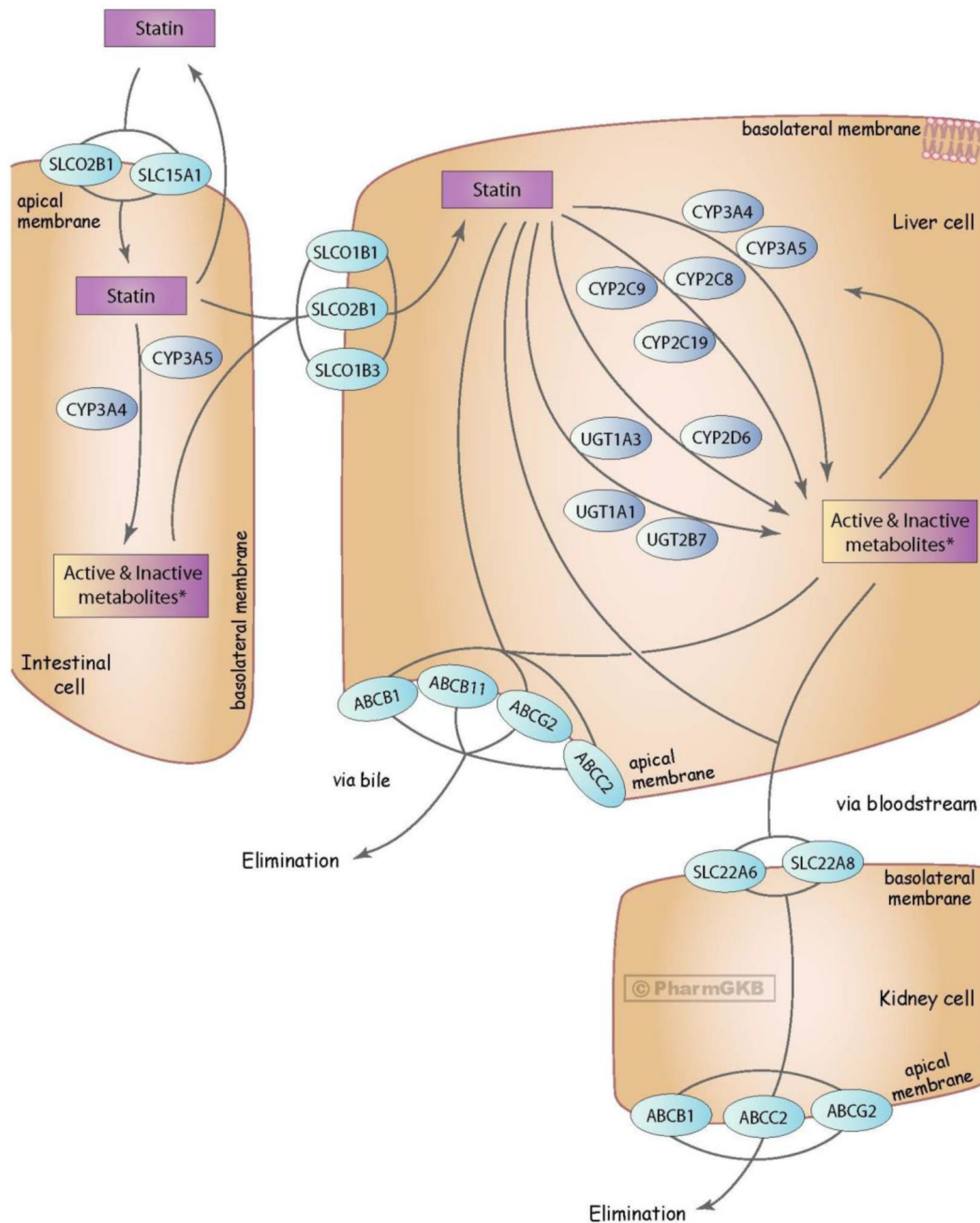
and T.E. Klein. From pharmacogenomic knowledge acquisition to clinical applications: the PharmGKB as a clinical pharmacogenomic biomarker resource. *Biomarkers in Medicine* 2011;5:795–806), and permission to reproduce it is provided by PharmGKB and Stanford University. PharmGKB©.

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**Figure 2. Genes involved in statin disposition**

Cellular uptake of statins is mediated by the *SLCO* and *SLC* gene families of transporters. Once inside cells, phase 1 metabolism of the drugs is mediated by *CYP* members, of which *CYP3A4* appears to be most important, in general. Phase 2 conjugation by glucuronosyltransferases (UGTs) is followed by cellular efflux by ABC cassette transporters. Specific details for individual statins is provided in the text, and a more detailed description of individual genes and gene products can be found at <http://www.pharmgkb.org/pathway/PA145011108> (accessed May 14, 2012). The figure is copyrighted by the Pharmacogenomics Knowledgebase (PharmGKB; E.M. McDonagh, M. Whirl-Carrillo, Y.

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**Table 1**

## Drug Distribution of FDA Approved Statins

Statin (year of approval)	Phase 0	Phase 1	Phase 2	Phase 3
Lovastatin (1987)	Passive diffusion OATP1B1 (minor)	3A4	UGT	BCRP
Simvastatin (1991)	Passive diffusion OATP1B1 (minor)	3A4	UGT	BCRP(lactone)
Pravastatin (1991)	OATP1B1	3A4 (minor)	UGT (minor)	MRP2 MDR1 (minor) BCRP (minor) BSEP (minor)
Fluvastatin (1993)	OATP1B1	2C9	?	BCRP
Atorvastatin (1996)	OATP1B1	3A4	UGT	BCRP
Rosuvastatin (2003)	OATP1B1 OATP1B3 (minor) OATP2B1 (minor)	3A4 (minor)	UGT (minor)	BCRP
Pitavastatin (2009)	OATP1B1 OATP1B3 (minor)	3A4 (minor)	UGT (minor)	MDR2 BCRP MRP2