Review article

Genetic polymorphisms as predictive markers for statin therapy: a route to improved cardiovascular patient outcomes?

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Cardiovascular disease (CVD) is a leading cause of morbidity and mortality worldwide. A significant risk factor for developing CVD is the presence of high plasma levels of low-density lipoprotein (LDL) cholesterol. With regard to pharmacological intervention, 'fat busting' statins are seen as the wonder drugs for lipid lowering and reducing the risk of myocardial infarction and stroke. However, there is wide inter-patient variability in measureable responses to these HMG-CoA reductase inhibitors, with individual patient genotypes being increasingly recognized as important contributors to this phenomenon. In recent years there have been great advances in our understanding of how personal genetics plays a role in controlling responses to drug therapies. Nevertheless, to date, there is no clinical application of identifying genetic markers that may predict responses to statins and subsequently modify cardiovascular outcomes. This review discusses the current literature regarding the potential roles of individual genetic polymorphisms in influencing responses to statins, and whether this can translate into clinical benefits for patients. While the significance of individual single nucleotide polymorphisms is yet to be established, it is suggested from genome-wide association studies that combinations of polymorphisms could be of greater clinical relevance. Further studies investigating the long-term influence of personal genetics on responses to statins and hard clinical outcomes are essential. As technology advances and the cost of genome sequencing falls, it will become increasingly easier to use individual genetic profiles to predict drug responses, tailor treatments and provide clinical benefit across populations.

Key words: statins, cardiovascular, genetics, pharmacogenetics, pharmacogenomics, biomarkers

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Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide (Balakumar, Maung and Jagadeesh, 2016). This umbrella term includes many common pathologies including myocardial infarction, stroke and coronary artery disease (CAD) (Nabel, 2003). Since CVD is so widespread, there is great demand for medication that lowers the risk of its development/progression. By far the most common drug class used in this way are the lipid-lowering statins, having a prescription prevalence of over 7 million in the UK alone between 1995 and 2013 (O’Keeffe, Nazareth and Petersen, 2016).

Having high plasma levels of low-density lipoprotein (LDL) is one of the main risk factors for developing CVD (Giner-Galvañ et al., 2016). LDLs are particles used to transport cholesterol and fatty acids in the blood (Eisenberg, 1984). Statins work by inhibiting the activity of the enzyme HMG-CoA reductase, the rate limiting step in endogenous
cholesterol synthesis (Stancu and Sima, 2001) (Fig. 1). A consequence of this action is the upregulation of LDL receptors in the liver, increasing LDL uptake and thereby decreasing plasma LDL levels (Brown and Goldstein, 1986, 1997). Importantly, the formation of mevalonic acid, a product of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity, is a precursor to other molecules as well as cholesterol. As such, statins have far wider ‘off target’ pleiotropic effects including the reduction of inflammatory and oxidative stress processes that drive atherosclerosis, and beneficial extra-cardiovascular effects on the immune system, central nervous system and bone (Bellosta et al., 2000; Liao and Laufs, 2005).

This statin-mediated reduction in LDL cholesterol has been shown to change hard outcomes such as death in numerous meta-analyses (Larosa, He and Vupputuri, 1999; Law et al., 2003; Unit, 2005; Yan et al., 2013; Wang et al., 2014). In a study of over 90 000 individuals, the relative risk of stroke and major coronary events was reduced by approximately 21% per mmol/l reduction in LDL cholesterol. Most statin regimes aim to achieve at least a 1.5 mmol/l decrease in LDL cholesterol, translating to >25 and 48 fewer major vascular events per 1000 participants for those taking statins for primary and secondary prevention, respectively (Unit, 2005). While statins are not appropriate for all patient groups, for example contra-indicated in expectant or breast feeding mothers, for the vast majority of the population they now provide first line LDL cholesterol reduction therapy. However, a confounding fact is that many patients do not experience these lipid-lowering effects, and indeed may suffer debilitating side effects.

**Statin resistance**

A marked inter-patient variation with regard to response to statin treatment has been observed, with up to nearly one half of those prescribed them not achieving their lipid reduction goals (Gitt et al., 2012). Moreover, even for those who do respond to statins, the time taken to achieve appropriate lipid lowering has high inter-participant variability (Reiner and Tedeschi-Reiner, 2013). As a consequence, many individuals remain at a much higher risk of cardiovascular events due to high LDL levels despite treatment intervention. The extent to which genotype plays a role in the patient response to statin therapy is currently poorly understood. Moreover, the pharmacokinetics of each statin differs widely, half-lives ranging from 1 to 19 h and oral bioavailability from 5% to 60% (Schachter, 2005). That said, a better knowledge of individual genotypes could have personal, and wider economical, benefits with regards to responsiveness to conventional statin therapy. Quick moving advances in personal high resolution technologies will in time make this an affordable reality.

**Benefits of using genetic polymorphisms as predictive biomarkers**

Pharmacogenetics is the study of inherited sequence variations in the deoxyribonucleic acid (DNA) code that can affect individual drug metabolism and subsequent therapeutic effects (Roses, 2000). This links up with pharmacogenomics which is more closely related to increasing the effectiveness of drugs by tailoring them to an individual genetic profile (Evans and Relling, 1999). As such it would seem straightforward to suggest that the use of such advances could help reduce the incidence of CVD thereby decreasing morbidity and mortality. Importantly, the economic benefits of such developments in personalized treatment are potentially enormous. CVD has significant cost implications, with the burden rising from around £1 billion in 2003–2010 to over £7.9 billion a year in the UK today (NICE, 2014). Increasing patient response to statin therapy, reducing the need for expensive secondary care, could lead to significant savings.

**Current use of genetics**

To date, neither The National Institute for Health and Care Excellence (NICE) or The Food and Drug Administration (FDA) recommend any predictive genetic screening before the commencement of statin therapy (FDA, 2011; NICE, 2014). However, the FDA does acknowledge the usefulness of such a strategy in recommending a 40 mg, rather than 80 mg, starting dose of simvastatin to reduce the risk of myotoxicity/myopathy (FDA, 2011). This adverse effect is a major barrier.
to effective cardiovascular risk reduction and ranges from increased plasma creatine kinase levels to fatigue, cramps, myalgia to life-threatening rhabdomyolysis (du Souich et al., 2017). Interestingly it would appear that single nucleotide polymorphisms (SNPs), by far the most common genetic variation found in humans (Consortium, 2010), may underlie this effect. Specifically the SNP rs4149056, found in the solute carrier organic anion transporter family member 1B1 (SLCO1B1) gene which encodes for the hepatic organic anion transporter P1B1 (OATP1B1), decreases the activity of this protein and results in markedly increased plasma concentrations of statins (Niemi et al., 2011). To this end a recent study demonstrated that there is a relative risk of statin-induced myopathy at 2.6 per copy of the C allele at rs4149056 for those taking a 10 mg dose of simvastatin, homozygotes (15% of the population) for the variant having an Odds Ratio (OR) of those variants found in HMG-CoA reductase (rs74739571) in HMG-CoA reductase that were related to a significant reduction in response to pravastatin therapy (Chasman et al., 2004). However, the absolute clinical relevance of this mutant allele needs to be confirmed by as yet unavailable studies relating to whether an increase in statin dose may counteract the reduced statin response or manifest in an increased side effect profile. Interestingly, and in stark contrast to that described above, the recent PROSPER TRIAL involving just under 6000 individuals concluded that there was no relationship between actions of statins and SNPs located on the HMG-CoA reductase gene, but instead found SNPs in the LDL receptors itself that influence the action of pravastatin (Poliscek et al., 2008).

### Candidate genes

Candidate genes are those already known to be involved in cellular/metabolic pathways (Paré et al., 2007), and in the case of response to statin treatment may include processes such as transcription/activity of HMG-CoA reductase and cellular drug transport.

### HMG-CoA reductase

One of the largest studies to investigate the role of SNPs in the statin/HMG-CoA reductase interaction identified two (rs74726928 and rs74739571) in HMG-CoA reductase that affected the action of pravastatin (Chasman et al., 2009). It is suggested that the drug reduces deposition pathways of statins (Elsby et al., 2012). ABCG2 (rs2231142) alteration of the pharmacokinetics of statin transport.

### ATP-binding cassette transporter C1 (ABCC1)

The ABCC1 gene codes for multidrug resistance-associated protein 1 (MRP1), a 17 transmembrane domain molecule that uses ATP hydrolysis to transport a wide range of toxic xenobiotic agents and endogenous material out of cells, against a concentration gradient, ready for excretion (Leslie et al., 2005; Reiner, 2014). Importantly, statins are eliminated from cells via this mechanism (Xu et al., 2005). A significant link between ABCC1 expression and LDL cholesterol reduction has been observed following atorvastatin therapy (Rebecchi et al., 2009). It is suggested that the drug reduces such as transcription/activity of HMG-CoA reductase and cellular drug transport.

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### Table 1. An example of other gene variants, beyond the focus of this review, but with known influence on statin metabolism

<table>
<thead>
<tr>
<th>Paper</th>
<th>Genes</th>
<th>Influence</th>
</tr>
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<tbody>
<tr>
<td>JUPITER trial (Chasman et al., 2012)</td>
<td>ABCG2 (rs2199936), LPA (rs10455872) and APOE (rs7412).</td>
<td>LDL reduction following rosvastatin therapy</td>
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<tr>
<td>CARDS study (Deshmukh et al., 2012)</td>
<td>LPA (rs10455872) and APOE (rs445925 and rs4420638).</td>
<td>Lower LDL response to atorvastatin</td>
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<tr>
<td>Heart protection study (Hopewell et al., 2013)</td>
<td>LPA, CELSR2/PSRC1/SORT1 and ABCC2</td>
<td>Novel lipid response to associated variants</td>
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<tr>
<td>Rooted in risk (Smit et al., 2016)</td>
<td>APOE, SORT1 and NPC1L1</td>
<td>Decreased LDL response to statin treatment</td>
</tr>
<tr>
<td>Deposition pathways of statins (Elsby et al., 2012)</td>
<td>ABCG2 (rs2231142)</td>
<td>Alteration of the pharmacokinetics of statin transport</td>
</tr>
<tr>
<td>Fluvastatin in relation to cytochrome P450 2C9 variant (Buzkova et al., 2012)</td>
<td>CYP2C9</td>
<td>Greater reduction in LDL on fluvastatin therapy</td>
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ABCC2, ATP-binding cassette sub-family C member 2; ABCG2, ATP-binding cassette sub-family G member 2; APOE, apolipoprotein E; CELSR2, cadherin EGF LAG Seven-Pass G-Type Receptor 2; CYP2C9, cytochrome P450 2C9; LDL, low-density lipoprotein; LPA, lipoprotein(a); NPC1L1, Niemann-Pick C1-Like 1; PSRC1, proline and serine rich coiled-coil 1; SORT1, sortilin 1.
ABCC1 gene expression, thus increasing the time statins can remain biologically active (Rebecchi et al., 2009). In contrast, the presence of SNPs can lead to overexpression of the protein, increased transport of the statins out of the cell and a reduction in their efficacy. Kajinami et al. concluded that the C3435T SNP in the ABCC1 gene was independently associated with poor responses to atorvastatin therapy in a gender specific manner (Kajinami et al., 2004). However, to date it would seem that there is still insufficient evidence for any clinical application of these findings (Reiner, 2014).

ATP-binding cassette transporter B1 (ABCB1)

The ABCB1 gene is a further member of the ABC transporter superfamily, and whilst its protein performs a similar function to the MRP1, it belongs to the separate P-glycoprotein subfamily (Hodges et al., 2011). It has been demonstrated that the C3435T SNP variant of ABCB1 is associated with a significantly smaller reduction in LDL cholesterol following both atorvastatin (Kajinami et al., 2004) and simvastatin (Fiegenbaum et al., 2005) therapy compared to non-carriers. Data from a further study suggests that the C3435T SNP is in linkage disequilibrium with the G2677T variant (Thompson et al., 2005) and therefore collectively influences statin pharmacokinetics (Soranzo et al., 2004).

Interestingly, the study by Mega and colleagues in 1500 post-actuate coronary syndrome patients found the non-GC mutant alleles of the G2677T/A and C3435T haplotype to be associated with an absolute 10.5% smaller pravastatin-induced reduction in LDL cholesterol levels compared to controls (Mega et al., 2009). However, as discussed earlier, one should bear in mind that individual statin pharmacodynamics may vary (Schachter, 2005). As such, it is difficult to generalize the findings of any one study using a specific statin and relate them to other drugs in the class. This is especially relevant when pravastatin, used in many of these studies, is not the first line drug of choice in current NICE guidelines. (NICE, 2014)

Genome-wide association studies

With the publication of the Human Genome Project in 2003, the identification of genetic variations that may relate to drug metabolism became much more viable (Collins et al., 2003). Genome-wide association studies (GWAS) allow the genetic make-up of an individual to be cross-referenced with that of the human genome sequence. Once enough individual data has been collected, the population frequencies of genetic variations can be identified and correlational links of candidate genes made with pathologies or deviations in drug metabolism.

In a 4000 participant GWAS study by Barber and colleagues, a strong association between statin-mediated changes in cholesterol and the SNP rs4420638 within introns 1–2 of the CLMN gene, known as the TaqIB genotype, was identified (Barber et al., 2010). The CLMN gene codes for calmin, a transmembrane protein found in various tissues including the hepatocytes, however its function is still currently unknown in the liver (Chatterjee and Horwitz, 2014). Patients who were homozygous for the SNP had a 3% smaller reduction in total cholesterol when taking simvastatin, pravastatin or atorvastatin, in comparison with those who were homozygous for the wild type variant (Barber et al., 2010). However, a significant criticism of the Barber study is that all 4000 participants were white males and other studies having shown distinct variations in SNP frequencies between races (Scott et al., 2010). In such cases it is obviously very difficult to draw conclusions that would be valid for a wider population.

A cohort design study in elderly subjects by de Keyser and colleagues identified two further SNPs, rs1532624 and rs533556 in the Cholesteryl Ester Transfer Protein (CETP) and Apolipoprotein A1 (ApoA1) genes respectively, that were associated with a significantly higher cholesterol concentration under statin therapy (de Keyser et al., 2011). Interestingly the study identified that these SNPs were likely to be in linkage disequilibrium with those in the TaqIB allele as described above. This supports the idea that there are genetic polymorphisms that can affect the actions of statins as a class, potentially making the presence of the former far more clinically relevant.

While the above studies seem to hold promise, others have failed to produce such convincing data. For example, a 13 000 participant meta-analysis investigating the effects of the TaqIB genotype on statin-induced cholesterol lowering, concluded that although it did lead to differences in cholesterol levels, it did not significantly influence the cholesterol-lowering action of pravastatin (46). Indeed the study concluded that the TaqIB genotype was in fact associated with a reduction in plasma high density lipoprotein (HDL) cholesterol (strongly associated with a decreased CVD (Linton et al., 2013)) independently of statin therapy (Boekholdt et al., 2005). Although both the above studies concluded that individuals with the TaqIB genotype were likely to be at a higher risk of CVD, they reached the conclusions through very different rationale. Further, more wide-ranging studies are required.

Clinical relevance of pharmacogenetic studies

It is difficult to compare the various studies because of the lack of standardization with regard to the statin used, time between initial therapy and lipid profile measurement, age, race, sex and outcomes. For example, within the statin class, lipophilic/hydrophilic properties of individual drugs vary greatly. As such cellular activity and cholesterol-lowering actions of these drugs will be affected by SNPs within transporter proteins in very different ways. Such challenges are significant hurdles in addressing the clinical utility of the findings in a general larger population.
A significant criticism of many studies that identify associations between response to statin therapy and the polymorphisms described above is the type of outcomes they base their findings on. The main aim of statin therapy is the reduction of LDL levels, which in turn reduces cardiovascular events and ultimately mortality. However, most pharmacogenetic studies assess changes in LDL levels over a relatively short time frame and not the hard outcomes described. While biochemical changes are easier to measure, the validity and clinical relevance of such soft outcomes is questionable and claims that certain SNPs affect statin therapy cannot be substantiated. Until a study is conducted to compare hard outcomes between those with and without variant alleles, no real claims can be made about the health impacts those SNPs may carry. To date, one of the few studies that used cardiovascular events as an outcome when investigating the effects of the CLMN gene SNPs on efficacy of statin therapy failed to establish a link between allele and hard outcome (de Grooth et al., 2004).

What then is the evidence that SNPs may be used as predictive markers for outcomes of statin treatment? A recent meta-analysis concludes that SNPs, such as those in the ABCB1 gene, can be used as pharmacogenomic biomarkers given they significantly effect outcomes (Su et al., 2015). Moreover, the largest meta-analysis to date involving SNPs, though only in those of European descent, supports this premise in demonstrating a link between HDL response and statin treatment (Postmus et al., 2016).

In stark contrast, a 2016 review that looked at the last 17 years of pharmacogenetic statin research concluded that although such genes as those described above do have associations with drug efficacy, the effect size is modest at best. Indeed, a proposed statin response change of 0.0394 mmol/l per allele mutation, translating to a 2% average change per patient, would appear to render genetic screening of little clinical value (Leusink et al., 2016). This point highlights the current debate comparing the values of traditional evidence-based and precision medicine. With new high resolution screening technologies it is possible to identify individuals who will not present with the responses to statin therapy that might be predicted by the evidence-based approach. Whether such a personalized perspective can actually translate to more favourable hard outcomes for these outliers remains to be seen (Beckmann and Lew, 2016).

Pseudo-tolerance

Despite promising genetic studies, a common reason patients do not achieve adequate statin-induced lowering of plasma cholesterol is ‘pseudo-tolerance’ as a result of treatment non-compliance (Bell et al., 2011). Regarding primary prevention, in their first year of treatment alone, it is predicted that 50% of patients stop taking statins as advised, with this number rising to 70% thereafter. In the case of secondary prevention of acute coronary events, such behaviour is almost as common with 40% non-adherence of treatment after 2 years (Jackevicius et al., 2002; Mann et al., 2010). Clearly patient education to emphasize the importance of compliance is essential. With regard to establishing the role of genetic influences on statin treatment, it will be essential to identify and minimize such artificial data in future detailed studies (Trompet et al., 2016).

Conclusion

For genetic screening to be beneficial for statin users, it would likely require the combined assessment of multiple genes to increase the likelihood of changing cardiovascular outcomes. It has been suggested that investigating 100 candidate genes at the same time could identify 15% of individuals who do not meet LDL lowering goals (Kim et al., 2014). Interestingly a recent study investigating the combined effects of 59 SNPs demonstrated that the lipid-lowering response was 3.4% less than that observed in control patients (Smit et al., 2016). It is evident that statins are seen by many as ‘Wonder Drugs’ with regard to preventing CVD. While the study of pharmacogenomics is in its infancy, such knowledge has already been successfully translated from bench to bedside for other medications such as immunosuppressants (Singer, 2016). Consequently, there is no reason why such an approach could not be used for statins in the future. As technology progresses the establishment of individual genetic profiles may further benefit those taking these drugs, adding to the economic benefits of a global reduction in cardiovascular morbidity and mortality.

Author biography

I am currently in my 5th year of study at Cardiff University reading Medicine. Last year, I undertook an intercalated BSc in Pharmacology which was a wonderful introduction to the world of academics and the science behind clinical medicine. I especially enjoyed learning about how personal genetics influences an individual’s health. The famous quote from the former GSK CEO ‘Most of our drugs don’t work in most people most of the time’ really made me think about the notion of personalized medication. An interest in cardiovascular pharmacology, and practical experience in the genetics laboratories during a summer placement, inspired me to write this review. I would very much like to remain in the research field of medicine during my clinical career and undertake a pharmacology-related PhD in the future.

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