ABCA1 mutation carriers are characterized by increased arterial stiffness

Submitted for publication

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Abstract

Objectives
Carriers of mutation in ATP-binding cassette transporter A1 (ABCA1) are characterized by life-long low plasma HDL-c levels and increased atherosclerosis. Evidence abounds on the role of ABCA1 in endothelial function in animal models, but no human data exist. Pulse wave velocity (PWV) is a reliable measure of arterial wall stiffness. PWV has been shown to be associated with ABCA1-mediated cholesterol efflux and endothelial function and constitutes an independent risk factor for cardiovascular disease. This prompted us to investigate whether PWV is increased in ABCA1 mutation carriers and to assess its correlations with carotid vessel wall thickness and HDL-c.

Methods
Lipid profiles, PWV and vessel wall thickness were assessed in 26 ABCA1 mutation carriers, 52 normolipidemic matched controls and 15 subjects with low HDL-c without underlying genetic defect.

Results
Plasma HDL-c was 42% lower in ABCA1 mutation carriers compared to normolipidemic controls. PWV was 21% higher in ABCA1 mutation carriers (p=0.007) compared to controls, independent of conventional risk factors. Furthermore, PWV correlated significantly with vessel wall thickness in both carriers and controls (p=0.002 and 0.007, respectively). PWV did not correlate with HDL-c (p=0.18 and 0.85, respectively).

Conclusions
This is the first evidence that ABCA1 mutation carriers are characterized by increased PWV, independent of HDL-c. This reflects their increased CHD risk and may constitute the first evidence of endothelial dysfunction in human ABCA1 mutation carriers. Given the correlations with vessel wall thickness, PWV may be a valuable, cost-effective, non-invasive addition to current CVD monitoring practice.
Introduction

Plasma high density lipoprotein (HDL) cholesterol is inversely correlated with coronary heart disease (CHD) risk.\(^1\) Carriers of mutation in the ATP-binding cassette transporter (ABC) A1 have dramatically decreased plasma levels of HDL-c. \(ABCA1\) mutation carriers have a loss-of-function of the \(ABCA1\) protein, which is responsible for the lipidation of lipid-poor apolipoprotein (apo) A1, and are characterised by increased atherosclerosis\(^2,3\) and CHD risk.\(^4,5\)

The increased CHD risk in \(ABCA1\) mutation carriers is thought to be attributable to decreased transport of vessel wall cholesterol to plasma HDL, a process known as reverse cholesterol transport (RCT).\(^6,8\) Indeed, homozygosity for \(ABCA1\) mutations, a condition known as Tangier disease, is characterized by foamy macrophages in both the vessel wall and other organs such as tonsils and spleen.\(^9\) Whether the pro-atherogenic phenotype in \(ABCA1\) mutation carriers is associated with functional changes of large arteries is unresolved to date.

Pulse wave velocity is a strong and independent predictor of CHD\(^10-12\) and constitutes the gold-standard for non-invasive measurement of arterial stiffness.\(^13\) PWV has emerged in recent years as a novel biomarker for predicting cardiovascular mortality and morbidity.\(^13\)

We have previously shown that PWV was increased in lecithin-cholesterol acyltransferase (\(LCAT\)) mutation carriers, another form of genetically low HDL-c, attributed to impaired RCT.\(^14\) Furthermore, a recent study reports an association between PWV and cholesterol efflux, as assessed by addition of participants’ plasma to an \(ABCA1\)-expressing cell line \textit{ex vivo}.\(^15\) Last, endothelial function is an important regulator of arterial stiffness. In animal models, Abca1 has been shown to play a crucial role in endothelial function.\(^16\) However, whether this translates into increased PWV in human \(ABCA1\) mutation carriers, remains to be established.

We compared PWV as a measure of arterial stiffness in carriers of loss of function mutations in \(ABCA1\) to normolipidemic controls and low HDL-c subjects. In addition, we assessed the association between PWV and carotid ultrasound measurements and carotid magnetic resonance imaging to determine its association with anatomical large artery changes. We hypothesized that \(ABCA1\) mutation carriers have increased PWV as compared to matched controls.

Methods

Study design

Subjects with low HDL-c levels, defined as HDL-c < 5\(^{th}\) percentile, were selected from a cohort of hypoalphalipoproteinemia patients\(^17\) and screened for \(ABCA1\) mutations. Family members of \(ABCA1\) mutation carriers were actively recruited. In order to limit referral bias, we excluded family probands who were referred to our outpatient clinic with clinically manifest CVD. Unaffected family members were asked to participate as controls. Since an insufficient number of family members consented to participation, the control group was complemented with unrelated controls recruited by advertisement. Carriers of functional \(ABCA1\) mutations (n=26) were matched one on two to controls (n=52) for age and gender. Twenty six \(ABCA1\) mutation carriers were furthermore compared to a low HDL control group of 15 individuals without underlying genetic defect, matched for age, gender and plasma...
HDL-c. All participants provided written informed consent. The study was conducted at the Academic Medical Center in Amsterdam, the Netherlands from March 2010 to November 2011. The study protocol was approved by the Institutional Review Board.

Presence of cardiovascular risk factors, use of medication and family history of CVD were assessed by a questionnaire. Body mass index (BMI) was calculated from weight and length. Blood was obtained after an overnight fast and stored at -80 °C. Plasma cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG) were analysed using commercially available kits (Randox, Antrim, United Kingdom and Wako, Neuss, Germany). Plasma apolipoprotein A1 and apolipoprotein B were measured using a commercially available turbidometric assay (Randox, Antrim, United Kingdom). All analyses were performed using the Cobas Mira autoanalyzer (Roche, Basel, Switzerland).

Genotyping
Mutation detection was performed as published previously. In short, the sequence reactions were performed using a BigDye terminator ABI prism kit (Applied Biosystems, Foster City, CA, USA). Sequences were analysed with the Sequencher package (Gene Codes Co, Ann Arbor, Mi, USA).

Blood pressure and PWV measurements
Participants visited the hospital after an overnight fast and were asked to refrain from smoking (if applicable) at least 3 hrs before the visit. All measurements were carried out in supine position after 15 min rest in a quiet, temperature-controlled room. The investigators were blinded for the genetic status of the participants. Brachial blood pressure was measured 3 times at 1- min intervals in supine position at the right arm after 15 min rest using a validated oscillometric device (Omron 705IT). The mean of the last 2 measurements was used for analysis. Measurements of carotid-femoral pulse wave velocity (PWV) were performed with the SphygmoCor system (Atcor Medical Pty Ltd, West Ryde, Australia). Pulse waveforms were recorded at the right carotid and femoral artery sequentially. Wave travel distance was calculated by subtracting ‘carotid artery - suprasternal notch distance’ from ‘suprasternal notch - femoral artery distance’. Measurements were conducted in duplicate and means were used for analysis.

Carotid ultrasound imaging
Carotid B-mode ultrasound scans of the left and right common, bulb and internal carotid arterial far walls were assessed as previously published. One reader analyzed all the images, blinded for group and any other data of the participants. The ultrasound parameter was mean common carotid intima-media thickness (CcIMT), defined as the average far wall IMT of the left and right distal 1cm of the common carotid artery.

Carotid magnetic resonance imaging
Scans were performed in a random subset of study participants as described previously. In short, scans were obtained in a 3.0 Tesla Philips whole-body scanner (Philips, Best, the Netherlands), using a single-element microcoil (Philips, Hamburg, Germany). Ten slices
were scanned of the distal 3.0 cm of the left and right common carotid artery. A total of 20 images were obtained per scan. Images were saved in DICOM format using standardized protocols. Quantitative image analysis was performed using semi-automated measurement software (VesselMass, Leiden University Medical Center, the Netherlands).

One trained reader, with excellent scan-rescan and intraobserver variability analyzed all the images using standardized protocols for reading and rating images combined with dedicated semi-automated software, blinded for all data of the participants. Lumen area (LA) and outer wall area (OWA) were measured. Normalized wall index (NWI) was calculated as: NWI= MWA / OWA. Mean wall area is calculated as: MWA=meanOWA-meanLA.

Statistical analysis

Continuous variables are expressed as means ± standard deviations (SD), unless otherwise specified. Possible differences in demographic, biometrical and biochemical parameters between carriers of ABCA1 mutations and controls were assessed using unpaired Student’s t-tests, Chi square tests or Mann-Whitney U-test, where appropriate. Differences in carotid imaging parameters between ABCA1 mutation carriers and controls were assessed using unpaired Student’s t-tests, unless otherwise specified. We chose to test correlations in the most conservative way, using nonparametric spearman’s rho coefficients and corresponding p-values. Difference between correlations were tested by means of a Fisher r-to-z transformation.

Results

Clinical characteristics

Baseline characteristics of study participants are listed in table 1. Twenty six ABCA1 mutation carriers from 14 separate families were included and compared to 52 normolipidemic controls, matched for age and gender, as well as 15 low HDL-c subjects. ABCA1 mutation carriers comprised two homozygous, two compound heterozygous and 21 heterozygous patients. Subjects were carriers of the following mutations: p.Leu1056Pro, c.3535+1G>C, c.6401+2T>C, p.Asn1800his, p.Phe1760Valfs*21, p.Cys1477Arg, p.Asp571Gly, p.Gln1038Ter, p.Thr929Ile, p.Arg587Trp, p.Asn935Ser and p.Arg579Gln. Cholesterol efflux impairment of all mutations has been previously published. CVD was more prevalent in ABCA1 mutation carriers than controls (p=0.03). Total cholesterol was 16% lower in ABCA1 mutation carriers (p=0.007), largely due to a 42% reduction in HDL-c (p<0.001), but was similar in low HDL-c subjects. Apolipoprotein AI was correspondingly decreased by 30% (p<0.001). Systolic blood pressure was higher in carriers compared to controls (p=0.04). Baseline characteristics were similar between ABCA1 mutation carriers and low HDL-c controls except for a trend towards higher triglycerides (p=0.07) and less males (p=0.09) in the low HDL control group.

Pulse wave velocity and vessel wall thickness in carriers of ABCA1 mutations and matched controls

PWV was increased in ABCA1 mutation carriers compared to controls (8.61±2.44 vs 7.14±1.34, p=0.007, table 2 and figure 1). Significance was retained after adjustment for
age, gender, BMI, history of CVD, statin use, smoking, diabetes and mean arterial pressure (p=0.007, figure 1). No gene-dose effect was observed when comparing homozygous and compound heterozygous ABCA1 mutation carriers to heterozygous mutation carriers or controls. After sensitivity analysis, excluding the two and three carriers with highest PWV values, significance was retained (p=0.02 and p=0.04 respectively). In order to discern an ABCA1 specific effect from an HDL-c effect, we also assessed PWV in subjects with low plasma HDL-c without an underlying genetic defect. PWV in ABCA1 mutation carriers did not differ from low HDL-c subjects (8.61±2.44 vs 7.98±1.80, p=0.39) (table 2).

Normalized wall index was increased in ABCA1 mutation carriers compared to normolipidemic controls (p=0.007) but not compared to low HDL-c controls (p=0.15),

### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n=52)</th>
<th>ABCA1 mutation carriers (n=26)</th>
<th>P1</th>
<th>Low HDL controls (n=15)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.6±11.7</td>
<td>49.7±16.2</td>
<td>0.34</td>
<td>48.7±10.8</td>
<td>0.84</td>
</tr>
<tr>
<td>Male sex, n (%)*</td>
<td>33 (64)</td>
<td>16 (62)</td>
<td>0.87</td>
<td>2 (13)</td>
<td>0.09</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>25.8±3.5</td>
<td>25.1±3.1</td>
<td>0.37</td>
<td>26.7±3.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Smokers, n (%)*</td>
<td>5 (10)</td>
<td>6 (23)</td>
<td>0.23</td>
<td>0</td>
<td>~</td>
</tr>
<tr>
<td>Diabetes, n (%)*</td>
<td>1 (2)</td>
<td>1 (4)</td>
<td>0.74</td>
<td>1 (8)</td>
<td>0.61</td>
</tr>
<tr>
<td>History of CVD n (%)*</td>
<td>2 (4)</td>
<td>5 (19)</td>
<td>0.03</td>
<td>2 (13)</td>
<td>0.63</td>
</tr>
<tr>
<td>Alcohol use n (%)</td>
<td>26 (79)</td>
<td>21 (81)</td>
<td>0.85</td>
<td>10 (83)</td>
<td>0.85</td>
</tr>
<tr>
<td>Statin use (%)*</td>
<td>6 (12)</td>
<td>7 (27)</td>
<td>0.09</td>
<td>5 (33)</td>
<td>0.66</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)#</td>
<td>129.8±12.4</td>
<td>136.5±13.4</td>
<td>0.04</td>
<td>135.4±11.6</td>
<td>0.79</td>
</tr>
<tr>
<td>Diastolic (mmHg)#</td>
<td>78.8±9.1</td>
<td>77.6±9.2</td>
<td>0.59</td>
<td>81.7±9.6</td>
<td>0.36</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>95.0±9.6</td>
<td>97.8±9.6</td>
<td>0.26</td>
<td>100±9.7</td>
<td>0.63</td>
</tr>
<tr>
<td>Hypertension, n (%)*</td>
<td>6 (13)</td>
<td>6 (25)</td>
<td>0.19</td>
<td>3 (27)</td>
<td>0.89</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.94±1.03</td>
<td>4.17±1.33</td>
<td>0.007</td>
<td>4.65±1.47</td>
<td>0.30</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.21±0.78</td>
<td>2.91±1.07</td>
<td>0.23</td>
<td>3.31±0.92</td>
<td>0.26</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.40±0.45</td>
<td>0.81±0.33</td>
<td>&lt;0.001</td>
<td>0.87±0.23</td>
<td>0.49</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)#</td>
<td>0.85 (0.69-1.38)</td>
<td>1.10 (0.72-1.59)</td>
<td>0.15</td>
<td>1.64 (1.38-2.39)</td>
<td>0.07</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dL)</td>
<td>108.11±24.54</td>
<td>111.61±40.69</td>
<td>0.67</td>
<td>113.10±26.45</td>
<td>0.91</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dL)</td>
<td>147.20±26.82</td>
<td>102.28±33.07</td>
<td>&lt;0.001</td>
<td>127.22±22.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are indicated as mean ± SD unless otherwise indicated. P-values are for student’s T-test unless otherwise indicated. P₁ compares carriers to controls; p₂ compares carriers to low HDL-C controls. * p for X² test. #: median and interquartile range; P for Mann Whitney U test.
while carotid ultrasound was not different between groups, which is in line with our earlier observations (p=0.62 and 0.64, respectively) (table 2).

Correlation between PWV and vessel wall thickness
PWV was significantly correlated with cIMT in carriers (p=0.62, p=0.003, figure 2a), as well as in low HDL-c controls (p=0.83, p<0.001) and normolipidemic controls (p=0.44, p=0.006, figure 2a). In line, PWV was significantly correlated with NWI in carriers (p=0.64, p=0.002, figure 2b) and normolipidemic controls (p=0.44, p=0.007, figure 2b). There was a trend towards a correlation between PWV and NWI in low HDL-c controls (p=0.54, p=0.07). There was no significant difference in the PWV-vessel wall thickness correlations between the groups.
Table 2. Pulse wave velocity and vessel wall thickness parameters in ABCA1 mutation carriers, normolipidemic controls and low HDL-c controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>ABCA1 mutation carriers</th>
<th>Low HDL controls</th>
<th>P^1</th>
<th>P^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV m/s</td>
<td>7.14±1.34</td>
<td>8.61±2.44</td>
<td>7.98±1.80</td>
<td>0.007</td>
<td>0.39</td>
</tr>
<tr>
<td>NWI</td>
<td>0.32±0.04</td>
<td>0.36±0.06</td>
<td>0.34±0.04</td>
<td>0.007</td>
<td>0.15</td>
</tr>
<tr>
<td>cIMT (mm)</td>
<td>0.66±0.15</td>
<td>0.64±0.20</td>
<td>0.64±0.11</td>
<td>0.62</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Values are indicated as mean ± SD. P-values are for student’s T-test. PWV is pulse wave velocity, NWI is normalized wall index, cIMT is carotid intima media thickness. MRI data were available in 36 controls, 21 carriers and 12 low HDL-c controls. cIMT data were available in 38 controls, 21 carriers and 12 low HDL-c controls.

Correlation between PWV and HDL-c

PWV was not associated with plasma HDL-c levels in carriers (ρ=0.28; p=0.18, figure 3), low HDL-c controls (ρ=-0.18, p=0.53) nor in normolipidemic controls (ρ=0.03; p=0.85, figure 3). There was no significance difference in the PWV-HDL-c correlations between the groups.

Discussion

In this study, we show that aortic pulse wave velocity is increased in ABCA1 mutation carriers compared to controls, indicating increased arterial stiffness in ABCA1 mutation carriers. In addition, PWV correlated strongly with measures of carotid artery wall thickness.

The increased arterial stiffness in ABCA1 mutation carriers is in line with the study by Favari and co workers, showing that PWV is inversely correlated with ABCA1 dependent cholesterol efflux, measured as cholesterol efflux from J774 macrophages to serum from study participants. However, whether PWV is increased in ABCA1 mutation carriers, was never investigated. Previously, we showed that PWV is also increased in another group of subjects with a defect in the reverse cholesterol transport pathway downstream of ABCA1.

In LCAT mutation carriers, esterification of nascent HDL particles is impaired, resulting in a lesser concentration gradient for cholesterol to flux from macrophage to HDL, resulting in more intracellular cholesterol. These findings may point to a detrimental role of intracellular cholesterol accumulation in vascular homeostasis, resulting in increased arterial stiffness.

The mechanism underlying increased PWV in ABCA1 mutation carriers may pertain to decreased endothelial function in response to reduced cholesterol efflux. In animal models, removal of the endothelium altered arterial stiffness. Terasaka and co workers showed that cholesterol accumulation in endothelial cells leads to impaired release of the vasodilator NO, due to an inhibitory interaction between caveolin 1 and endothelial NO synthase (eNOS). In line, blocking NO synthesis in humans increases arterial stiffness. Westerterp and co workers subsequently published that cholesterol accumulation in endothelial cells is associated with increased atherosclerosis in mice, possibly linking endothelial function to atherogenesis. In humans, endothelial function is inversely associated with arterial stiffness, suggesting that the increased PWV in ABCA1 mutation carriers is a reflection...
Figure 2. correlation between PWV and vessel wall thickness. a. correlation between PWV and intima media thickness (carotid ultrasound). b. correlation between PWV and normalized wall index (carotid MRI). a. carotid-femoral PWV plotted to carotid IMT. Continuous line indicates correlation between PWV and carotid IMT in controls (open symbols, n=38). Dashed line indicates correlation between PWV and carotid IMT in carriers (closed symbols, n=22). b. carotid-femoral PWV plotted to normalized wall index (NWI). Continuous line indicates correlation between PWV and NWI in controls (open symbols, n=36). Dashed line indicates correlation between PWV and NWI in carriers (closed symbols, n=22).

due to cellular cholesterol accumulation. In line with this hypothesis, the slope of the correlation between NWI and PWV appeared to be steeper in ABCA1 mutation carriers than in controls, suggesting that arterial stiffness is caused by ABCA1 specific vascular alterations. This is further supported by the finding that there was no significant correlation between PWV and the most sensitive parameter of vessel wall thickness, MRI. Furthermore, the strong correlation between NWI and PWV was not found in for example Fabry patients, who are not characterized by decreased RCT. Alternatively, atherosclerosis has been suggested to directly cause arterial stiffness by inducing alterations
in the vessel wall. Our findings that NWI is increased in ABCA1 mutation carriers and strongly associates with PWV may be interpreted as supportive of this concept.

Interestingly, PWV did not correlate with plasma HDL-c levels. This is in line with the finding by Favari and co workers, that PWV correlates with ABCA1 mediated cholesterol efflux, independently of plasma HDL-c. Furthermore, this is in line with the absence of a difference between ABCA1 mutation carriers and low HDL-c controls. In addition, we and others have shown that in a cohort of LCAT mutation carriers, PWV was also found to be associated with vessel wall thickness, but not with plasma HDL-c levels. This notion supports the hypothesis that intracellular cholesterol accumulation due to ABCA1 deficiency underlies decreased endothelial function, resulting in increased PWV.

The finding that PWV was higher in ABCA1 mutation carriers, characterized by low plasma HDL-c, but did not correlate with plasma HDL-c concentration is intriguing. In line, PWV was not different in low HDL-c subjects compared to normolipidemic controls. Our limited sample size hampers definite conclusions, but our findings support the concept that ABCA1 deficiency and not plasma HDL-c levels underlies increased PWV. The finding of increased PWV in low HDL-c subjects compared to normolipidemic controls can be explained by the fact that cardiovascular risk factors are likely to be present in low HDL-c subjects without underlying genetic defect, whereas in ABCA1 mutation carriers, low HDL-c may be attributable to genetic deficiency alone.

Interestingly, no gene-dose effect was observed when comparing PWV in homozygous ABCA1 mutation carriers to heterozygous ABCA1 mutation carriers. This is likely due to the small sample size of homozygous/compound heterozygous carriers en may furthermore reflect the propensity of physicians to do everything within their means to reduce CVD risk in these patients with near-absent HDL-c. Alternatively, compensating mechanisms may have occurred in response to life-long exposure to extremely low HDL-c levels.
Several aspects of our study merit closer consideration. The sample size is relatively small, inherent to the low prevalence of ABCA1 mutation carriers in the general population. However, an important strength of our study is the individual matching of ABCA1 mutation carriers to controls. Although not significant, the percentage of males is lower in the group of low HDL-c subjects. PWV did not differ between males and females in any of our groups, indicating that gender did not significantly influence PWV in our cohort. Furthermore, systolic blood pressure was higher in ABCA1 mutation carriers compared to controls. However, the increased PWV in carriers retained significance after correction for systolic blood pressure, indicating that the increased PWV in ABCA1 mutation carriers is not attributable to the increased systolic blood pressure. Finally, the cross-sectional design of this study precludes us from answering whether increased PWV is causal or secondary to atherogenesis.

**Conclusion**

In this study, we provide the first evidence for the concept that ABCA1 mutation carriers are characterized by increased PWV, independent of HDL-c. This reflects their increased CHD risk and may constitute the first evidence of endothelial dysfunction in human ABCA1 mutation carriers. Given the correlations with vessel wall thickness, PWV may be a valuable, cost-effective, non-invasive addition to current CVD monitoring practice.

**Perspectives**

PWV is a reproducible, non-invasive and readily applicable proxy of vessel wall condition. Especially given its strong correlations with measures of vessel wall thickness in both study groups, the present work supports it to be a valuable technique to assess and monitor CVD risk in high risk patients. Furthermore, the finding that PWV is increased in ABCA1 mutation carriers, possibly due to a direct ABCA1-related effect on the vessel wall, opens the door for specific interventions aiming at specific ABCA1 upregulation in order to improve endothelial function. Given the finding that with conventional CVD risk reduction a substantial residual risk of 65-75\% remains, implicates that a large population may benefit from such interventions. PWV would be the pre-eminent parameter for monitoring such therapy.

**Acknowledgements**

We are indebted to all study participants. The authors would like to thank C.A.M. Koch and J.F. Los for their assistance in expanding the pedigrees and J. Peter for his role in identification of ABCA1 mutation carriers,

**Sources of funding**

This work was supported by the Dutch Heart Foundation (grant numbers 2008B070 and 2009B027). Andrea Bochem is supported by fellowship WdL/HE/12-029 from the Saal van Zwanenbergstichting, the Netherlands.

**Conflicts of interest**

None.
Reference List


