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DNA Damage and Repair in Patients With Coronary Artery Disease: Correlation With Plaque Morphology Using Optical Coherence Tomography (DECODE Study)

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ABSTRACT

Objective: The aim of this study was to examine DNA ligase activity and expression of DNA damage response pathway (DDR) genes in patients with stable angina (SA) and non-ST elevation myocardial infarction (NSTEMI) and determine whether they correlate with plaque morphology.
Background: Patients with coronary artery disease (CAD) have evidence of deoxyribonucleic acid (DNA) damage in peripheral blood mononuclear cells (PBMCs). It is unclear whether this represents excess damage or defective DNA repair activity.
Methods: DNA ligase activity and the expression of 22 DDR genes were measured in PBMCs of patients (both SA ($n = 47$) and NSTEMI ($n = 42$)) and in age and gender-matched controls ($n = 35$). Target lesion anatomical assessment was undertaken with frequency domain optical coherent tomography.
Results: DNA ligase activity was different across the three groups of patients (control $= 119 \pm 53$, NSTEMI $= 115.6 \pm 85.1$, SA $= 81 \pm 55.7$ units/g of nuclear protein; ANOVA $p = 0.023$). Pair wise comparison demonstrated that this significance is due to differences between the control and SA patients ($p = 0.046$). Genes involved in double strand break repair and nucleotide excision repair pathways were differentially expressed in patients with SA and NSTEMI. In SA patients, fibrocalcific plaques were strongly associated with GTSE1, DDB1, MLH3 and ERCC1 expression. By contrast, in NSTEMI patients the strongest association was observed between fibrous plaques and ATM and XPA expression.
Conclusion: PBMCs from patients with CAD exhibit differences in DNA ligase activity and expression of DDR genes. Expression levels of certain DDR genes are strongly associated with plaque morphology and may play a role in plaque development and progression.
Trial Registration Number
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1. Introduction

Atherosclerotic coronary artery disease (CAD) and its complications remain the most common cause of morbidity and mortality in the Western World [1]. Both atherosclerotic plaques and circulating leukocytes in patients with CAD show markers of deoxyribonucleic acid (DNA) damage and activation of the DNA damage response pathway (DDR) suggesting that DNA damage may contribute to atherogenesis [2–4]. DNA damage is seen in early atherosclerotic lesions and becomes almost universal in advanced plaques [5]. However, it is unclear whether elevated DNA damage reflects increased exposure to damage-causing agents, defective DNA repair, or both. Furthermore, DNA repair activity and how it correlates with plaque morphology has not been studied in CAD patients.

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A major cause of DNA damage in atherosclerosis is oxidative stress [6]. The DNA repair pathways most pertinent to oxidative stress are the double strand break repair (DSBR) and base excision repair (BER) pathways. The importance of DNA repair activity, which may be measured in vitro using DNA ligase assays, in atherosclerosis is illustrated by premature aging disorders such as Werner syndrome whereby a deficiency in the WRN protein required for DSBR and BER leads to accelerated aging and atherosclerosis [7]. In addition, cultured plaque vascular smooth muscle cells (VSMCs) exhibit differential activation of 22 genes from multiple DNA damage and repair pathways, but most notably from those involved in BER and DSBR [8].

The aims of this study were to: (a) measure DNA repair using DNA ligase activity as well as the expression of a set of 22 DDR gens in PBMCs from patients with stable and unstable CAD, and (b) determine whether differences in DNA repair and/or gene expression correlate with plaque morphology as assessed by frequency domain optical coherence tomography (FD-OCT).

2. Methods

The DNA Damage and Repair in Patients with Coronary Artery Disease (DECODE) study was a prospective study approved by the Research Ethics Service Committee South East Coast-Surrey, UK (REC Reference: 13/LO/0238, IRAS Project ID: 120221) and is registered at ClinicalTrials.gov (NCT02335086). All participants gave written informed consent before enrolment. Consecutive patients undergoing percutaneous coronary intervention (PCI) for symptomatic stable angina (SA) despite optimal medical therapy or non-ST elevation myocardial infarction (NSTEMI) were prospectively enrolled. Age and gender-matched patients being investigated for chest pain and who were found to have normal coronary arteries upon coronary angiography were also recruited and served as the control arm of the study. Exclusion criteria were age ≥ 80 years, inability to provide informed consent, presentation with ST-elevation myocardial infarction, decompensated heart failure, left ventricular ejection fraction ≤ 35%, prior coronary revascularization (surgical or percutaneous), diabetes mellitus, peripheral arterial disease, previous history of cerebrovascular disease, any malignancy, or active inflammatory disorders, and anatomical conditions precluding FD-OCT such as significant tortuosity, and severe calcification. FD-OCT was performed prior to culprit lesion PCI following maximal vessel dilatation with intra-coronary glyceryl trinitrate. A Dragonfly Duo FD-OCT imaging catheter (Abbott Vascular ILUMIEN OPTIS PCI Optimisation system, California, USA) was used utilising a 2.7-French monorail delivered through a 6-French guide catheter over a standard 0.014-inch intra-coronary guidewire. This rapid exchange monorail-imaging catheter was connected to a central console containing the light source, image processing software and imaging display (ILUMIEN OPTIS PCI Optimisation system). The image analysis was performed off-line using QCU-CMS software (LKEB, Leiden, The Netherlands). The region of interest included at least 30 mm of the most proximal acquired coronary segment. Frames at 1 mm intervals were analysed. Each frame was characterized according to one of the following plaque types: normal vessel (no plaque), fibrous plaque, fibrocalcific and fibroatheroma. The latter was further categorized into thin (fibrous cap ≤ 65 μm) or thick-cap fibroatheroma (>65 μm).

Blood was taken from the arterial sheath immediately before PCI and PBMC isolated using the Optiprep™ gradient centrifugation as previously described [9]. Nuclear protein extracts were prepared from PBMCs using the NE-PER™ Nuclear and Cytoplasmic Extraction Reagents Kit, according to manufacturer's guidelines (ThermoFisher Scientific, Massachusetts, USA) and protein concentrations determined using the BCA Protein Assay kit (ThermoFisher Scientific, Massachusetts, USA). Nuclear DNA repair activity was measured using a microplate-based DNA ligase assay. DNA ligase and DNA polymerase repair activities were quantified using microplate-based assays, one in which double stranded oligonucleotide substrates containing either a single ligatable strand nick or a strand nick with a single nucleotide gap immobilised on the surface of the wells were repaired by the enzyme present in the extracts. Serial dilutions of recombinant T4 DNA ligase or exonuclease minus Klenow fragment (Promega) in the presence of excess T4 DNA ligase were used to construct standard curves from which the nuclear extract activities were interpolated. There was a strong correlation between activities determined with the two assays. Since the gap filling assay measures the combined repair activity of DNA polymerase and DNA ligase whereas the ligase assay only measures the latter, such a correlation suggests that either expression of the DNA ligase and polymerase activity is closely coordinated or that the ligation step is rate limiting in the gap-filling assay. Spiking of

Table 1
Baseline patient characteristics. NSTEMI = Non-ST elevation myocardial infarction; CAD = Coronary artery disease; LV = Left ventricle; ACE = Angiotensin converting enzyme; ARB = Angiotension-2 receptor blocker.

Variables	Stable Angina (n = 47)	NSTEMI (n = 42)	Control (n = 35)	*p* Value
Age (years)	62.6 ± 6.7	58.1 ± 12.1	57.8 ± 9.7	0.08
Male Sex	34 (72.3%)	33 (78.6%)	20 (57.1%)	0.13
Body Mass Index (kg/m^2)	27 ± 4.2	27.7 ± 5.9	27.7 ± 4.6	0.75
Smoker	19 (40.4%)	29 (69%)	13 (37.1%)	0.008
Hypertension	29 (61.7%)	22 (52.4%)	8 (22.9%)	0.003
Family history of CAD	24 (51.1%)	20 (47.6%)	8 (22.9%)	0.03
History of Hyperlipidaemia	28 (59.6%)	12 (28.6%)	7 (20%)	<0.001
Haemoglobin (g/L)	14.2 ± 0.98	14.5 ± 1.3	14.3 ± 1	0.6
White cell count (×10^9/L)	7.3 ± 1.7	8.7 ± 2.8	7.1 ± 1.6	<0.001
Platelet count ((×10^9/L)	246 ± 62.3	233.9 ± 55.8	253.9 ± 53.6	0.32
Glomerular filtration rate (ml/min/1.73 m^2)	>60	>60	>60	1.0
Glucose (mmol/L)	5.2 ± 0.8	5.4 ± 0.9	5 ± 0.6	0.16
Total cholesterol (mmol/L)	4.5 ± 1.2	4.7 ± 1.2	5.1 ± 0.8	0.07
Non-HDL cholesterol (mmol/L)	3.13 ± 1.05	3.6 ± 1.0	3.5 ± 0.8	0.15
Triglyceride (mmol/L)	1.4 ± 1.1	1.5 ± 1.2	1.6 ± 0.9	0.85
LV ejection fraction	62.2 ± 7.2%	62.1 ± 4.5%	63.6 ± 3.6%	0.47
Medication				
Aspirin	47 (100%)	42 (100%)	11 (31.4%)	<0.001
Thienopyridine	47 (100%)	42 (100%)	1 (2.9%)	<0.001
Beta blocker	40 (81.6%)	38 (90.5%)	7 (20%)	<0.001
ACE inhibitors	32 (65.3%)	40 (95.2%)	7 (20%)	<0.001
A2RB	7 (14.3%)	2 (4.8%)	0 (0%)	0.03
Statins	47 (100%)	42 (100%)	9 (25.7%)	<0.001
Calcium channel antagonist	12 (24.5%)	2 (4.8%)	6 (17.1%)	0.04

Table 2
Angiographic, procedural characteristics, and FD-OCT based measurements of plaque morphology. The OCT data are presented as mean ± one standard deviation. NSTEMI=Non-ST-elevation myocardial infarction; AHA = American Heart Association. Plaque categories are the percentages of the plaque categories along each subject's vessel averaged over all subjects.

Variable	Stable Angina (n = 47)	NSTEMI (n = 42)	p value
Culprit vessel			
Proximal left anterior descending	15 (31.9%)	8 (19%)	0.21
Mid left anterior descending	18 (38.3%)	19 (45.2%)	0.35
Circumflex artery	6 (12.8%)	2 (4.8%)	0.20
Right coronary artery	10 (21.3%)	13 (31%)	0.26
AHA lesion classification			
A	17 (36.2%)	12 (28.6%)	0.54
B	31 (66%)	30 (71.4%)	0.41
C	1 (2.1%)	0 (0%)	0.32
Syntax score	7.7 ± 4.4	10.3 ± 5.6	0.03
Plaque category (%)			
Normal vessel	26.6 ± 21.7	23.0 ± 19.6	–
Thin capped fibroatheroma	17.0 ± 10.4	26.4 ± 20.5	–
Thick capped fibroatheroma	25.3 ± 16.6	24.7 ± 17.0	–
Fibrous plaque	38.1 ± 17.5	39.0 ± 26.4	–
Fibrocalcific plaque	26.8 ± 26.0	23.4 ± 25.3	–

nuclear extracts samples with excess exonuclease minus Klenow fragment DNA polymerase had no substantial effect on the apparent gap-filling activity whereas spiking extracts with extra DNA ligase dramatically enhanced the apparent gap filling activity. This suggests that the correlation observed was because the DNA ligase activity in the nuclear extracts was the rate-limiting step of the two. Consequently, only the DNA ligase activity data are presented.

Gene expression analysis by quantitative reverse transcription polymerase chain reaction (qRT-PCR) for 22 human DDR genes was outsourced to Qiagen (Hilden, Germany). These genes have previously been found to exhibit the most consistent and significant differential expression in cultured human VSMCs and atherosclerotic plaques ex-vivo from an original 84 gene human DDR pathway array. All FD-OCTs were analysed with blinded patient's background at the Medstar Cardiovascular Research Network Core Laboratory, MedStar Washington Hospital Centre, DC (USA) using internationally recognised guidelines [10,11].

In the absence of published data regarding parameters such as DNA ligase activity in humans with and without atherosclerosis, a sample size of 30 patients per group was recommended to detect an effect size of 0.9 with a power of 80% and 2-tailed α of 0.05. In total, 124 were recruited to comfortably allow for three cohorts of at least 30 patients. Statistical analysis was performed using SAS, version 8.2 (SAS Institute, Cary, North Carolina). Continuous variables and categorical variables are expressed as mean ± standard deviation respectively. Clinical demographics were analysed using a 1-way Analysis of Variance (ANOVA) or 2-tailed unpaired *t*-test with Welch correction as appropriate. DNA ligase activity and gene expression data were analysed using a 1-way ANOVA. Bonferroni's correction was used for pair wise comparisons for control versus SA, control versus NSTEMI, and SA versus NSTEMI. The gene expression data were compared using the student's t-test and the q-value calculated using Benjamini and Hochberg method. This q-value gives the minimum False Discovery Rate (FDR) at which the test may be called significant. Differences were considered significant for q-values <0.05. Correlation between FD-OCT plaque parameters and gene expression/DNA ligase activity was analysed using Spearman rank correlation. Hierarchical cluster analysis of the correlation matrix was used to assess associations of the rows (gene expression) and columns (plaque parameters). A Heatmap overlaid on the clusters displays the rows and columns as rectangles scaled along a colour gradient indicating the strength of associations.

3. Results

The baseline characteristics of the three groups of patients are summarized in Table 1. The three groups differed in the rates of smoking, hypertension, family history of CAD, history of hyperlipidaemia, total white cell count, and secondary prevention medication, which were

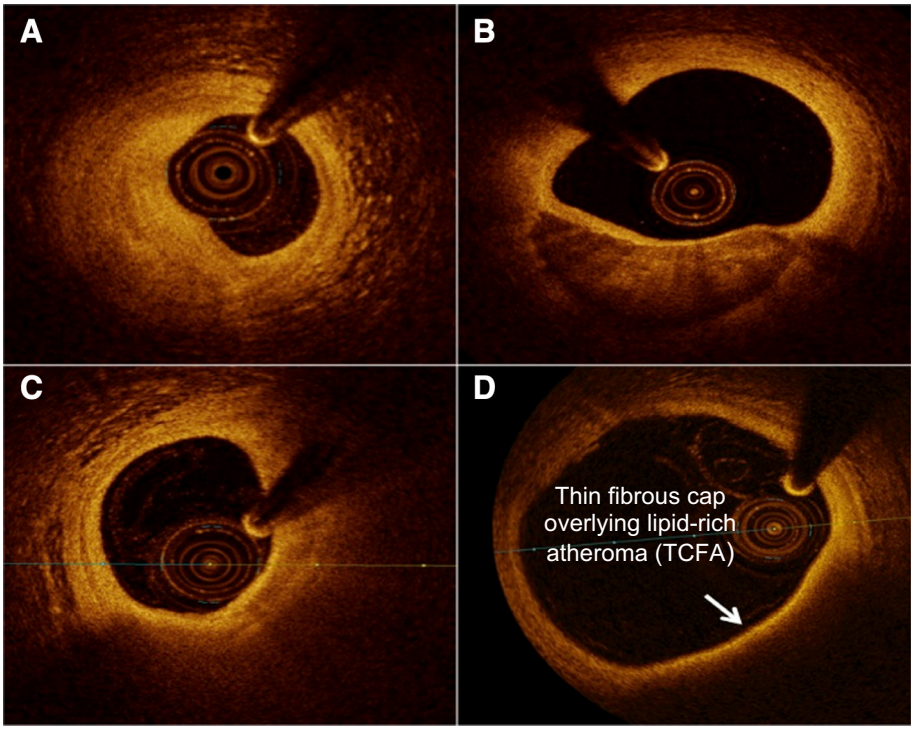

Fig. 1. Examples of atherosclerotic plaques visualised by frequency domain optical coherent tomography (FD-OCT). Fibrotic (A), Fibrocalcific (B), lipid rich (C), and thin-cap fibroatheromatous (D) lesions are identified.

all increased in CAD patients. All SA and NSTEMI patients were on statins but a greater proportion of NSTEMI patients were on an angiotensin converting enzyme inhibitor (ACEI).

The angiographic and procedural characteristics as well as the OCT parameters are summarized in Table 2. The culprit vessel and the American Heart Association lesion classification were similar in the two groups. The SYNTAX score was low in both groups but significantly higher in NSTEMI patients (10.3 ± 5.6 vs. 7.7 ± 4.4; $p = 0.03$). The FD-OCT parameters measured included lipid-rich plaque, which has been further characterized into thin or thick-cap fibroatheroma depending on the fibrous cap thickness (65 μm was used as the criteria with measurements <65 μm defining a thin-cap fibroatheroma), fibrous plaque and fibrocalcific plaque (Fig. 1).

The mean DNA ligase activity was different across the three groups of patients (control = 119 ± 53, NSTEMI = 115.6 ± 85.1, SA = 81 ± 55.7 units/g of nuclear protein; ANOVA $p = 0.023$) (Fig. 2). Pair wise comparison demonstrated that this significance arose due to differences between the control and SA patients ($p = 0.046$). There was no correlation between ligase activity and age ($r = -0.094$), total cholesterol ($r = 0.013$), or total white cell count ($r = 0.027$).

As compared to controls, patients with SA demonstrated significant down-regulation of genes involved in the DSBR pathway (BRCA1, RBBP8, RAD50, XRCC6), nucleotide excision repair pathway (XPA, DDB1, and CDK7), transcriptional mediators such as ABL1 and SUMO1, and up-regulation of OGG1, involved in BER, MLH3, involved in mismatch repair, and GADD45A, a p53 target (Table 3). As compared to control subjects, NSTEMI patients had down-regulation of RAD50, CDK7, as well as SUMO1 and up-regulation of OGG1 (Table 4). Heatmaps of DDR gene expression are shown in Figs. 3 and 4. The Heatmap indicates that in patients with SA, fibrocalcific plaques are strongly associated with GTSE1, DDB1, MLH3 and ERCC1 expression. By contrast, in NSTEMI patients the strongest association was observed between ATM and XPA with fibrous plaques.

4. Discussion

This study demonstrates proof of concept that patients with CAD exhibit alterations in DNA repair and DDR gene expression, some of which are associated with plaque morphology. This is the first description to our knowledge of such findings in patients with CAD.

Accumulating data over the past two decades has implicated the DDR as an essential feature of atherosclerosis [12,13]. For example in rabbit models of atherosclerosis, DNA strand breaks normalized within weeks of statin therapy but 8-oxo-G immunoreactivity persisted significantly longer implying that DNA repair is inefficient in atherosclerosis [12]. Similarly, apolipoprotein-deficient mice that were haploinsufficient for ATM have also been shown to exhibit persistent DNA damage and failure to repair DNA resulting in defects in cell proliferation, apoptosis and mitochondrial dysfunction [13]. By contrast, there is limited data on DNA repair in human atherosclerosis. PBMCs isolated from patients with SA and acute coronary syndromes showed evidence of DNA damage including shortened telomeres and mitochondrial DNA damage [14]. Monocytes from these patients showed

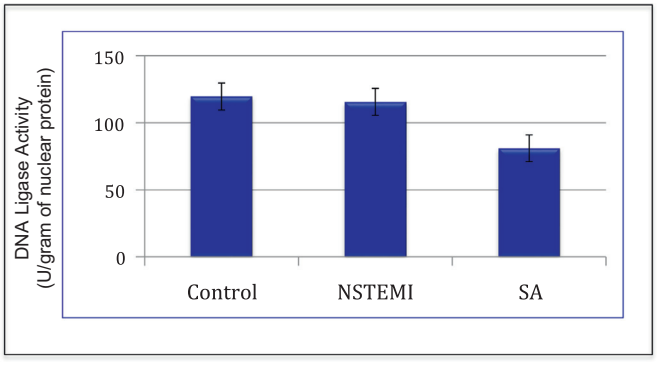

Fig. 2. DNA ligase activity in patients with stable angina (SA), non-ST elevation myocardial infarction, as well as age and gender-matched controls (ANOVA = 0.023). Pair wise comparison demonstrated that this significance arises due to differences between the control and SA patients ($p = 0.046$).

Table 3
Comparison of gene expression in control and stable angina patients. CI LB = Lower bound of the 95% Confidence interval; CI UB=Upper bound of the 95% Confidence Interval. As compared to controls, patients with SA demonstrated significant down-regulation of genes involved in the DSBR pathway (BRAC1, RBBP8, RAD50, XRCC6), nucleotide excision repair pathway (XPA, DDB1, and CDK7), transcriptional mediators such as ABL1 and SUMO1, and up-regulation of OGG1, involved in BER, MLH3, involved in mismatch repair, and GADD45A, a p53 target.

Probe ID	Control			Stable			q-Value
	Mean	CI LB	CI UB	Mean	CI LB	CI UB	
CDK7	16.3	15.0	17.6	12.1	11.3	12.8	<0.001
RAD50	15.9	14.3	17.4	11.3	10.3	12.4	<0.001
MLH3	26.5	20.6	32.4	51.9	43.8	60.1	<0.001
DDB1	65.5	62.1	68.9	55.0	52.0	58.0	<0.001
OGG1	7.8	7.1	8.5	11.0	9.9	12.1	<0.001
SUMO1	31.8	30.0	33.5	27.6	26.1	29.0	<0.001
ABL1	9.1	8.2	10.0	7.5	6.9	8.2	<0.001
RBBP8	5.6	5.1	6.1	4.5	4.0	5.0	0.01
BRCA1	2.6	2.3	2.8	2.0	1.8	2.3	0.01
GADD45A	0.6	0.5	0.8	1.1	0.8	1.4	0.01
XPA	3.5	3.0	4.0	2.8	2.4	3.2	0.04
XRCC6	30.9	28.8	33.0	28.5	26.9	29.9	0.09
MRE11	16.9	15.3	18.5	14.9	13.5	16.4	0.11
CHEK1	1.5	1.2	1.8	1.2	0.9	1.5	0.16
GTSE1	0.3	0.1	0.4	0.5	0.3	0.6	0.18
PRKDC	4.5	4.0	5.0	4.0	3.6	4.5	0.18
ATM	22.1	19.4	24.9	19.8	17.9	21.7	0.18
NLRP2	3.1	2.1	4.1	3.0	2.4	3.6	0.9
ERCC2	3.0	2.6	3.4	3.0	2.7	3.3	0.92
EBNA1BP2	22.0	20.5	23.6	22.3	20.3	24.2	0.92
TP73	0.1	0.1	0.2	0.1	0.1	0.2	0.93
ERCC1	14.2	13.1	15.4	14.1	13.0	15.3	0.94

Table 4
Comparison of gene expression in control and non-ST elevation myocardial infarction (NSTEMI) patients. CI LB = Lower bound of the 95% Confidence interval; CI UB=Upper bound of the 95% Confidence Interval. In patients with NSTEMI, we found down-regulation of RAD50, CDK7, and SUMO1 and up-regulation of OGG1.

ProbeID	Control			NSTEMI			q-Value
	Mean	CI LB	CI UB	Mean	CI LB	CI UB	
CDK7	16.3	15.0	17.6	13.1	12.3	13.9	<0.001
OGG1	7.8	7.1	8.5	10.5	9.1	11.8	0.01
RAD50	15.9	14.4	17.4	13.3	12.2	14.3	0.03
SUMO1	31.7	30.0	33.5	28.6	27.1	30.1	0.04
MLH3	26.5	20.6	32.4	38.8	30.7	47.0	0.074
ABL1	9.1	8.2	9.9	7.9	7.3	8.5	0.75
XPA	3.5	3.0	3.9	3.0	2.7	3.3	0.18
TP73	0.1	0.1	0.2	0.1	0.1	0.1	0.20
ERCC2	3.0	2.6	3.4	3.4	3.1	3.7	0.20
ATM	22.1	19.4	24.9	19.4	17.6	21.2	0.20
ERCC1	14.2	13.1	15.4	15.4	14.2	16.6	0.34
MRE11	16.9	15.3	18.5	15.7	14.3	17.1	0.47
GADD45A	0.6	0.5	0.8	0.7	0.6	0.8	0.50
RBBP8	5.6	5.1	6.1	5.2	4.6	5.8	0.50
CHEK1	1.5	1.2	1.8	1.3	0.9	1.8	0.63
GTSE1	0.3	0.1	0.4	0.3	0.2	0.4	0.63
NLRP2	3.1	2.1	4.1	3.6	2.6	4.7	0.62
EBNA1BP2	22.0	20.5	23.6	21.5	20.3	22.7	0.71
BRCA1	2.6	2.3	2.8	2.5	2.2	2.7	0.72
DDB1	65.5	62.1	68.9	64.5	61.1	67.8	0.72
PRKDC	4.5	4.0	5.0	4.5	4.1	4.9	0.88
XRCC6	30.9	28.8	33.0	31.0	29.3	32.6	0.96

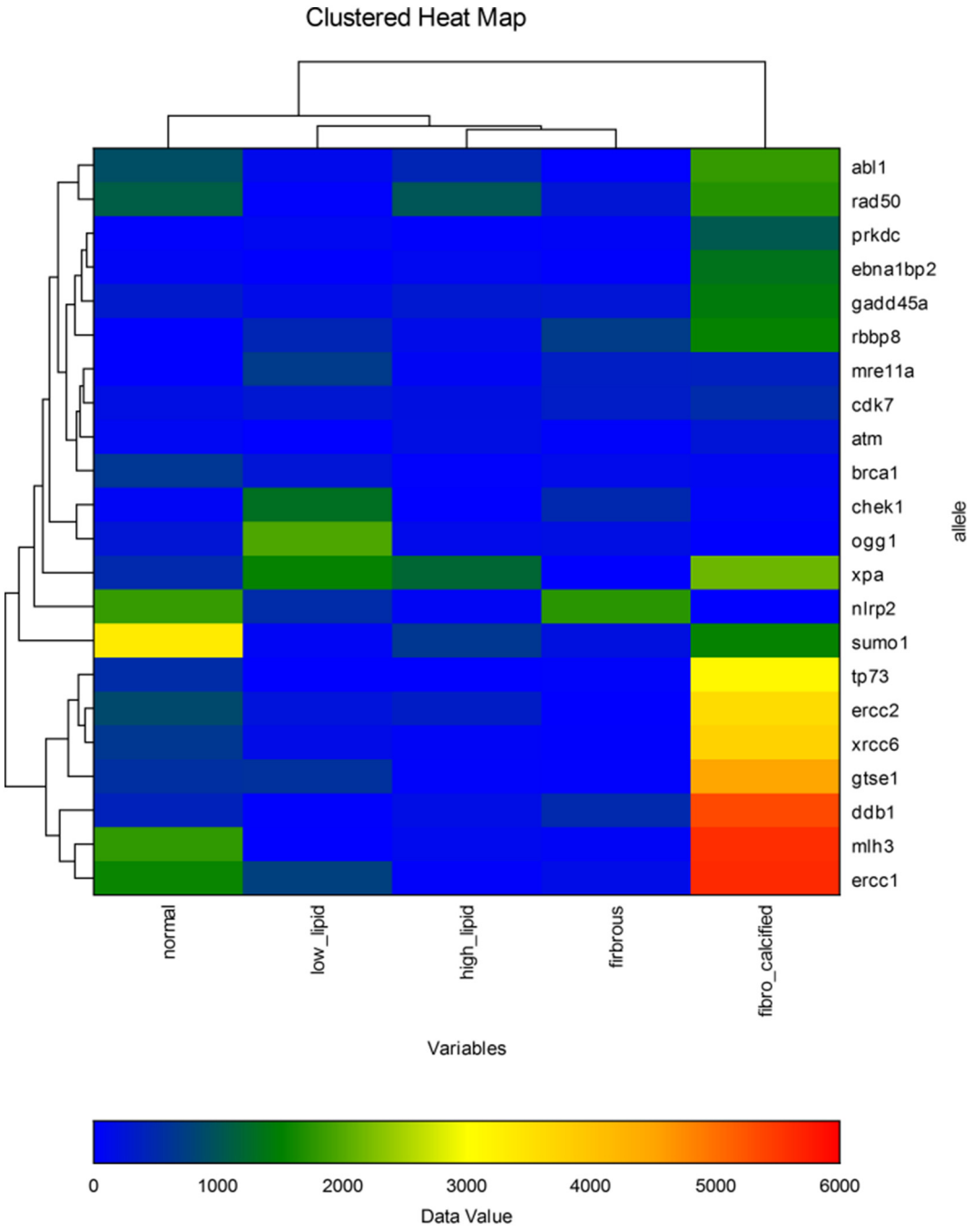

Fig. 3. Clustered Heatmap in patients with stable angina. There are strong associations between GTSE1, DDB1, MLH3 and ERCC1 expression and fibrocalcific plaques. Low_lipid = thin-cap fibroatheroma; High_lipid = Thick-cap fibroatheroma.

increased basal and lipopolysaccharide–induced cytokine release (MCP-1, IL-6, and IL-1ß) and increased production of reactive oxygen species indicating that DNA damage may promote a pro-inflammatory monocyte/macrophage phenotype. Indeed, DNA damage using the telomere-disrupting agent telomestatin reproduced this phenotype and both telomere shortening and mitochondrial DNA damage were correlated with thin-cap fibroatheroma indicating that DNA damage in peripheral circulating cells may promote an unstable plaque phenotype [14].

Cultured human aortic VSMCs have been shown to exhibit robust changes in the expression of 22 DDR genes [8]. We therefore elected to study the expression of these genes in our study. As compared to control subjects, we find that patients with SA exhibit a wide range of abnormalities in the expression of DDR including reduced expression of genes implicated in DSBR and NER, as well as increased expression of certain genes involved in BER and transcriptional mediators such as SUMO1. GTSE1, MLH3, and ERCC1 were strongly associated with fibrocalcific plaques. This bears remarkable resemblance to the published in vitro data [8]. DNA ligase activity was also significantly reduced in SA patients. By contrast, patients with NSTEMI had alterations in the expression of RAD50, CDK7, SUMO1 and OGG1. ATM and XPA expression was strongly associated with fibrous plaques. Collectively, these results indicate that the contribution of DDR gene expression and DNA ligase activity to the atherosclerotic process may be different in patients with SA and NSTEMI. Post-mortem studies have indicated that 60–70% of myocardial infarctions are caused by rupture of thin-capped

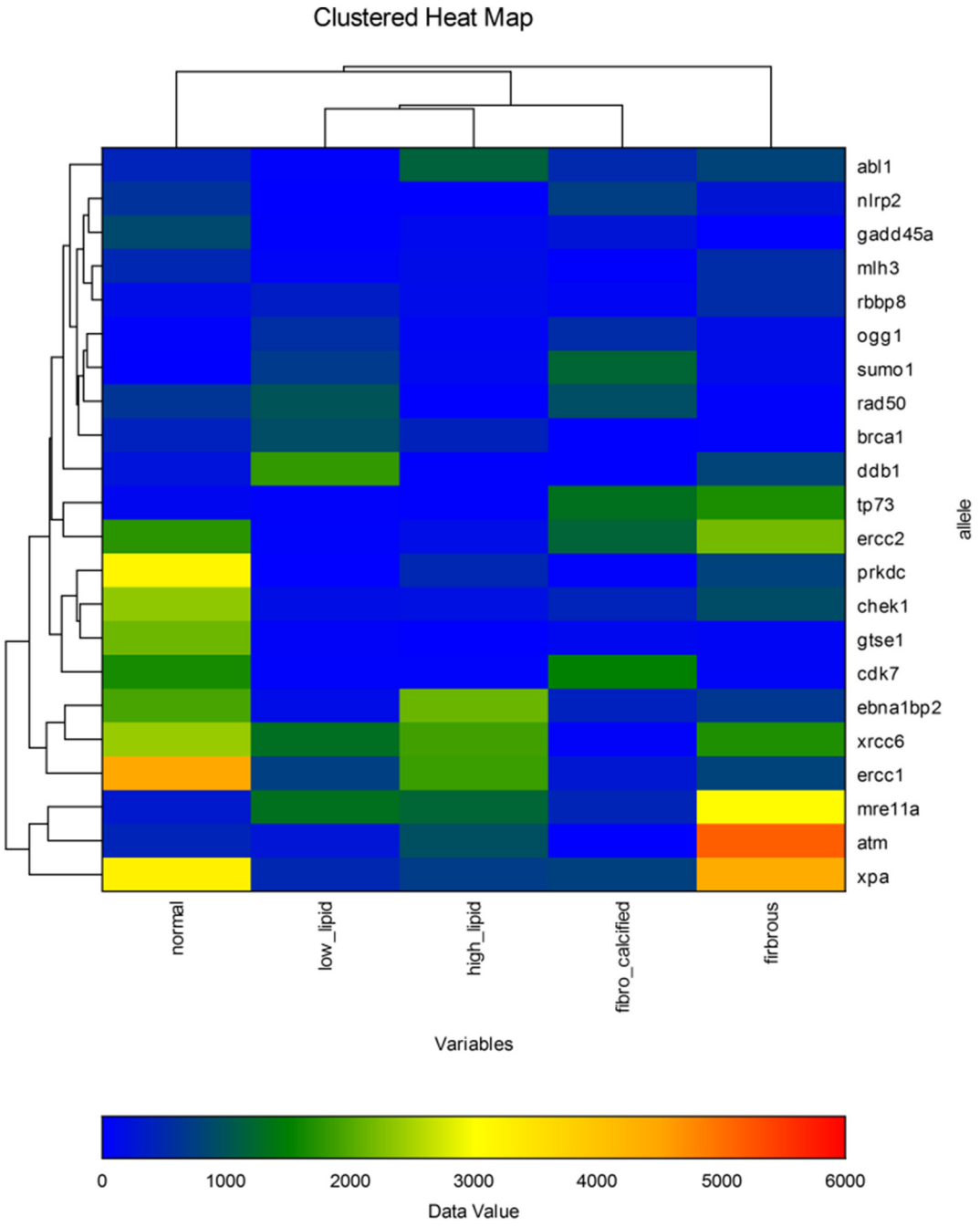

Fig. 4. Clustered Heatmap in patients with non-ST elevation myocardial infarction. There are strong associations between ATM and XPA expression and fibrous plaques. Low_lipid = thin-cap fibroatheroma; High_lipid = Thick-cap fibroatheroma.

fibroatheromata in which a thin cap containing macrophages separate the necrotic core from the lumen, often without significant vessel stenosis [15]. Furthermore, such lesions tend to have larger necrotic cores, and fewer VSMCs than lesions in SA. We speculate that differences in plaque composition between SA and NSTEMI as well as differences in the inflammatory status of such patients may, at least in part, be responsible for differences in DNA repair activity and the expression profiles of genes involved in the DDR. One caveat to this argument is the higher proportion of NSTEMI patients who were on ACEI. This family of drugs have been shown to reduce the production of reactive oxygen species and consequent DNA damage through telomere-dependent and non-telomeric pathways [3].

In addition to differences in ligase activity and the expression of DDR genes, we have found associations between the expression of such genes and plaque morphology. Crosstalk between plaque and the circulation have been noted in other studies such as PROSPECT in which circulating CRP concentrations predicted the rate of major adverse coronary events (MACE); 13.8% of thin-cap fibroatheromatous non-culprit lesions were linked to MACE in those with CRP concentrations > 10 mg/l compared with only 1.9% in patients with CRP concentrations < 3 mg/l [16]. A number of studies have indicated that DDR genes have important roles in the development of atherosclerosis. For example, OGG1(−/−) Ldlr(−/−) mice fed a Western diet developed increased plaque size and lipid content [17]. The plaques contained increased oxidized mitochondrial DNA, inflammasome activation, and apoptosis whilst there were elevations in serum IL-1ß, and IL-18. Restoration of OGG1 status protected against these changes and reduced plaque size. OGG1 has also been shown to be a key regulatory

enzyme for 8-oxoguanine repair in VSMCs with OGG1 (−/−) mice showing increased levels of 8-oxoguanine in vivo and increased atherosclerosis [18]. Furthermore, ATM (+/−) ApoE (−/−) mice develop accelerated atherosclerosis and features of the metabolic syndrome including hypertension, hypercholesterolemia, obesity, and impaired glucose tolerance [13]. Smooth muscle cells and macrophages from such mice showed increased nuclear DNA damage, defective DNA repair, growth arrest and apoptosis.

There are a number of limitations to our study. Firstly, we have measured nuclear DNA ligase activity in PBMCs rather than individual cellular components, in particular peripheral monocytes. Although this may have been more informative, lymphocytes, monocytes, and neutrophil activity are all implicated in atherosclerosis. Secondly, our study is descriptive and does not provide a mechanism by which DNA ligase activity and DDR gene expression contribute to atherosclerosis. Thirdly, as our patient cohort had a low SYNTAX score we cannot be certain that our findings can be extrapolated to those with intermediate and high SYNTAX scores. However, it is likely that such patients may exhibit a more pronounced alteration in DNA ligase activity and genes involved in the DDR.

5. Conclusion

In conclusion, we found that patients with CAD exhibit alterations in their ability to repair DNA damage and DDR gene expression. Such alterations may contribute to the development and progression the atherosclerotic process.

Disclosure of Competing Interest

None of the authors have any conflicts of interest, financial or personal, related to the contents of this manuscript.

Author contribution

Drs Shah, Meira, Elliott, Bennett, Curzen, and Mahmoudi have contributed to the conception and design of the study as well as data analysis and interpretation. Drs Hoole, West, Brown, Garcia-Garcia, Kuku, Kazuhiro, Kolm, and Mariathas have been involved in data analysis and interpretation. All authors have been involved in drafting the manuscript, revising it critically for important intellectual content, and final approval of the submitted manuscript. None of the authors have any relationship with industry relevant to the contents of this paper.

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