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FINAL REPORT
(2015)

1. PROGRAMME AND PROJECT LEADER INFORMATION

	Research Organisation Programme leader	ARC Research Team Manager	Project leader
Title, initials, surname	Dr DP van Velden		Prof MJ Kotze
Present position	Senior lecturer		Researcher
Organisation, department	Division of Anatomical Pathology, University of Stellenbosch, Tygerberg		Division of Anatomical Pathology, University of Stellenbosch, Tygerberg
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2. PROJECT INFORMATION

Research Organisation Project number	N09 08-225 2012
Project title	Analysis of the relationship between diet and genetic risk factors with a special emphasis on the effects of alcohol consumption on health and disease
Short title	

Fruit kind(s)	Grapes		
Start date (mm/yyyy)	01/2010	**End date** (mm/yyyy)	12/2015

Key words	Alcohol, wine, brandy, genotype, health benefits

Approved by Research Organisation Programme leader (tick box) ☐

3. EXECUTIVE SUMMARY

Objectives & Rational

The cholesterol-raising properties of the apolipoprotein E (APOE) epsilon-4 (ε-4) allele has been validated in the South African population. Mounting evidence supports the added value as a cardiovascular risk marker in dyslipidemic patients

beyond its established use to improve diagnostic reliability for late-onset Alzheimer's disease (AD). The aim of this study is to determine the appropriateness of combining family history with non-genetic information relevant to APOE genotyping as an integral component of a chronic disease screening program.

Methods

A total of 580 South African individuals prospectively enrolled in a chronic disease screening program incorporating a genetic component (2009-2014) were selected for inclusion in this study based on the presence (75) or absence (505) of a family history of AD. Biochemical assessment of serum lipid profiles was performed according to standard laboratory protocols, and all study participants were genotyped for the APOE ε-2/ε-4 alleles using allele-specific TaqMan real-time polymerase chain reaction technology.

Key Results

In patients without a family history of AD, APOE genotype modified the relationship between alcohol intake and body mass index ($p=0.026$), with a significant positive correlation noted between these parameters being limited to ε-4 allele carriers. APOE genotype modified the association between alcohol intake and total serum cholesterol in patients with ($p=0.026$) and without ($p=0.048$) a family history of AD.

Conclusion/Discussion

The assessment of non-genetic data including AD family history and alcohol intake could assist in determining eligibility for APOE genotyping used to facilitate the avoidance of environmental risk in a genetic subgroup of dyslipidemic patients.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

Cardiovascular disease (CVD) and Alzheimer's disease (AD) share overlapping pathogenic mechanisms and common genetic risk factors. It is in this context that apolipoprotein E (APOE) polymorphisms provide a genetic link between the metabolic syndrome, vascular disease and dementia. The cholesterol-raising epsilon-4 (e-4) allele of the APOE gene is considered an important determinant of inter-patient heterogeneity in AD. Despite the strong association between the e-4 allele and shared disease risk across diagnostic boundaries, APOE genotype has limited individual utility as a diagnostic, predictive and prognostic marker in the context of cardiovascular risk screening and management. A lack of existing referral guidelines for APOE genotyping presents an important limitation which impedes the more widespread application of such testing as a routine component of cardiovascular risk screening. Recognition hereof led to the development of a local database resource for translational research linked to a genomics-based chronic disease screening program incorporating APOE genotyping (Kotze et al. 2015).

This database facilitated recent local research aimed at determining whether the assessment of non-genetic information could assist in identifying patients set to derive optimal benefit from APOE genotyping performed in this context. In a previous local study, Lückhoff et al. (2015) indeed demonstrated that the genotype distribution for APOE ε-4/ε-2 differs between South African individuals with and without a family history of AD. In this particular study, the clinical expression of a hypercholesterolemic phenotype in ε-4 allele carriers, as well as its mitigation by regular physical activity, was shown to be dependent on the interaction

between APOE genotype and a family history of AD. In addition, the relationship between dietary saturated/trans-fat intake and serum lipid profiles was also dependent on the presence or absence of a self-reported family history of AD (Lückhoff et al. 2015). These observations collectively support the relevance of clinical inquiry concerning AD family history as part of a pre-screen algorithm used to determine eligibility for APOE genotyping, shown to add additional value in the management of dyslipidemic patients beyond its established role in improving diagnostic reliability for late-onset AD (Sun et al. 2012).

As an extension of the abovementioned findings, we aimed to determine the appropriateness of combining family history with non-genetic information relevant to APOE genotyping as an integral component of chronic disease risk screening and management. In particular, we sought to replicate the modifying influence of APOE genotype on the association between modifiable lifestyle habits and cardio-metabolic risk traits as demonstrated in previous international studies (Lindsay et al. 2002; Ruitenberg et al. 2002; Mukamal et al. 2003; Harwood et al. 2010) in relation to a self-reported family history of AD. Insight gathered as a result of this study could provide the scientific rationale supporting a multidisciplinary approach to cardiovascular risk screening, incorporating APOE genotyping to inform clinical and therapeutic decision making in the context of FH case finding, cardio-metabolic risk reduction and AD prevention.

5. DETAILED REPORT

a. PERFORMANCE CHART (for the duration of the project)

Milestone	Target Date	Extension Date	Date completed
1. Recruitment of study participants	October 2015		July 2015 (ongoing)
2. Data collection and captured in database	December 2015		July 2015
6. Journal publication(s) – final milestone See under 6d	December 2015		July 2015 (ongoing)

b) WORKPLAN (MATERIALS AND METHODS)

A non-interventional, cross-sectional genotype-phenotype association study was performed. Ethical approval for the study was granted by the Health and Research Ethics Committee (HREC) of Stellenbosch University under project number N09/08/224. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Written informed consent for participation in the research component of a chronic diseases screening program was obtained from all study participants.

Selection of Study Participants

Data of 580 self-reported Caucasian individuals of European Ancestry (404 females, 176 males, mean age = 46±12 years) prospectively enrolled in a chronic disease screening program incorporating a genomics component following referral by a private practicing clinician or attendance of Wellness Days at the workplace or other venues over a five year period (2009-2014) were available in a centrally maintained database (accessed at

www.gknowmix.org) linked to the research component of a pathology-supported genetic testing service. This group included all 537 participants (93%) enrolled in our previous study (Lückhoff et al. 2015) as well as an additional 43 eligible individuals referred for inclusion in the abovementioned screening program since April 2014. Full lipid profiles were determined for a subgroup of 291 patients (50%) included in the present study. To exclude ethnicity as a potential confounder, and given the small number of non-Caucasian individuals who provided informed consent for research, comparative analyses were performed only in the Caucasian study group, who were selected based on the presence (75) or absence (505) of a family history of AD.

Questionnaire-Based Clinical and Lifestyle Assessment
Prior to enrolment in the chronic disease screening program, all prospective study participants were asked to complete the ethically approved study questionnaire (available at www.gknowmix.com) developed in collaboration with a registered dietician and approved by the HREC at Stellenbosch University. This questionnaire was used to document socio-demographic information, clinical data such as body mass index (BMI) and lifestyle factors (dietary fat intake, alcohol consumption). In patients who reported a positive family history of AD, the relation to the affected individual was also documented in addition to age of onset. Alcohol intake was differentiated into abstain, occasionally (1- 2 units/week), moderate (1-13 units/week) and high (14 or more units/week). This corresponded to the ordinal categories 0-3 considered for statistical analyses. Study participants were also asked how frequently they ate certain foods rich in saturated or trans-fats over the course of the preceding three months. This was used to derive a dietary saturated and trans-fat score, considered to be moderate when ranging between 22 and 26, low between 16-21, very low if <16, and high if ≥27.

APOE Genotyping
Genomic DNA was extracted from whole blood (QIAamp® DNA Midi-kit) or saliva samples (Oragene® reagents). Conventional polymerase chain reaction (PCR) followed by direct DNA sequencing was performed for detection of the APOE ε-2 (rs7412) and ε-4 (rs429358) alleles including five internal control DNA samples. DNA sequencing resulted in the identification of the wild type, heterozygous and homozygous genotypes for the polymorphic variants of interest. These samples were used for analytical validation of high-throughput genotyping in our laboratory using TaqMan® SNP Genotyping Assays (Applied Biosystems, Werterstadt, Germany) performed on the Corbett Rotor-Gene™ 6000 series Multiplexing System.

Statistical Analysis
All statistical analyses were performed using the R Studio software package (freely available from http://www.r-project.org). The clinical characteristics of participants included in the validation phase of this study were described and compared between individuals with and without a family history of AD. Qualitative characteristics were described using cross-tabulation and frequency tables and compared between study groups using the Pearson's Chi-squared or Fisher's exact test as appropriate. Quantitative phenotypes were described as the means along with standard deviations (SD) and com-pared between study subgroups using a Student's t-test assuming normality of distribution. In cases where quantitative outcomes showed a non-symmetrical distribution, such variables were log-transformed for analyses. If the best-fitting log-transformed model still showed a non-normal distribution, these were described as the median with interquartile range (IQR) and compared between study groups using the Wilcoxon signed rank test. The linear relationship between the fat score and metabolic risk phenotypes was assessed using the Spearman rank correlation coefficient test, for the total study groups as well as subgroups defined based on the presence or absence of an AD family history. Analysis of covariance (ANCOVA) was used to determine whether APOE genotype modified the association between dietary habits and

metabolic risk phenotypes. A p-value of <0.05 was seen as statistically significant with trend-associations noted where applicable.

c) RESULTS AND DISCUSSION

In the total Caucasian study group, smoking was associated with higher triglyceride levels ($p<0.001$) and lower HDL cholesterol ($p=0.004$). No significant association between smoking status and BMI ($p=0.110$), total ($p=0.167$) or LDL cholesterol levels ($p=0.457$) was otherwise noted. In study participants with a negative family history of AD, the association between smoking and triglycerides ($p=0.002$) as well as HDL cholesterol levels ($p=0.022$) retained significance. No significant relationship between smoking and the selected metabolic risk phenotypes of interest were noted for study participants with a positive family history of AD ($p>0.05$).

In the total Caucasian study group, a trend was observed for alcohol intake to be positively associated with total ($p=0.070$) as well as LDL cholesterol levels ($p=0.071$). A statistically significant inverse association was identified between alcohol intake and HDL cholesterol ($p=0.009$). Alcohol consumption was not otherwise associated with BMI ($p=0.603$) or triglyceride levels ($p=0.449$) in the total study group. A trend towards a positive association between alcohol intake and total cholesterol was also noted for study participants with a negative family history of AD ($p=0.082$). In this subgroup, alcohol intake was not associated with BMI ($p=0.587$), LDL ($p=0.121$), HDL cholesterol ($p=0.201$) or triglyceride levels ($p=0.182$). In study participants with a positive family history of AD, a positive association was noted between alcohol intake and BMI ($p=0.008$) as well as triglyceride levels ($p=0.021$). In this subgroup, alcohol intake was not otherwise associated with total ($p=0.560$), LDL ($p=0.219$) or HDL cholesterol levels ($p=0.138$). In summary, the association between alcohol intake and metabolic risk phenotypes differed based on the presence or absence of AD family history.

No significant modifying influence for APOE genotype on the association between lifestyle habits and cardio-metabolic risk traits was noted when the total study group was considered. A number of differential effects were however observed when participants were stratified based on the presence or absence of a self-reported family history of AD. Firstly, APOE genotype modified the association between alcohol intake and total cholesterol in study participants with ($p=0.026$) and without ($p=0.048$) a family history of AD, with a significant positive association between these parameters being limited to ε-4 allele carriers (Figures 1A and B). In study participants with a negative history of AD, APOE genotype also modified the association between alcohol intake and BMI ($p=0.026$) with a significant positive correlation between these variables again being limited to ε-4 allele carriers (Figure 2). A trend was also noted for APOE genotype to modulate the relationship between alcohol intake and HDL cholesterol ($p=0.056$) with an inverse association being restricted to ε-4 allele non-carriers.

In this study, we provide additional evidence substantiating our previous finding that the modifying influence of APOE genotype on the association between lifestyle habits and selected cardio-metabolic risk phenotypes is influenced by AD family history. APOE ε-4 allele carriers did not derive the same cardio-protective benefits from moderate alcohol intake, and were more susceptible to the deleterious effects of excess alcohol consumption on serum lipid profiles. The role of AD family history as a determinant of genotypic effects evident from this study was deemed in keeping with our previous observation that its interaction with APOE genotype influences the clinical expression of a hypercholesterolemic phenotype in ε-4 allele carriers (Lückhoff et al. 2015). These findings therefore collectively support the relevance of lifestyle-based assessment positioned alongside clinical inquiry concerning AD family history as part of a multidisciplinary approach to chronic disease risk screening incorporating APOE genotyping. The added value of genetic testing in this context lies not only in prioritizing the need for lifestyle-based intervention aimed at decreasing cumulative

cardio-metabolic risk in a genetic subgroup of dyslipidemic patients, but also improving the identification as well as guiding the management of South African patients with FH.

Findings from our study collectively support the assessment of socio-demographic information as well as modifiable lifestyle habits as part of a pre-screen selection step used to determine the appropriateness of APOE genotyping performed as part of a multidisciplinary approach to cardiovascular risk management. In this context, genetic testing could prove useful in identifying a high-risk subgroup of non-FH dyslipidemics set to derive the greatest benefit from the timely implementation of lifestyle-based intervention strategies aimed at decreasing cumulative cardio-metabolic risk to prevent the onset and progression of AD in later life. In particular, emphasis is placed on the role of physical activity in normalizing serum lipid profiles and related metabolic abnormalities in APOE ε-4 allele carriers, as well as mitigating the deleterious effects of advancing age on multiple biochemical disturbances associated with dementia risk (Okonkwo et al. 2014). In this context, the favourable effects of exercise in maintaining normal cognition and decreasing the rate of cognitive decline are known to be more pronounced in APOE ε-4 carriers (Bernstein et al. 2002; Podelwis et al. 2005; Obisesan et al. 2012; Farina et al. 2014).

In summary, we showed that APOE ε-4 genotype modifies the association between alcohol and selected metabolic risk phenotypes, with a tendency for the adverse effects of these lifestyle habits to be exacerbated in risk-allele carriers. In addition, our findings suggest that the differential expression of these effects is itself influenced by the presence or absence of a family history of AD. This notion is in accordance with findings reported in a recent local study (Lückhoff et al. 2015) in which we demonstrated that the interaction between APOE genotype and AD family history determines the expression of a hypercholesterolemic phenotype in ε-4 allele carriers. Findings from these two studies support the use of a pre-screen algorithm incorporating clinical and lifestyle-based assessments to determine the appropriateness of APOE genotyping performed as an integral component of cardiovascular risk evaluation. A multidisciplinary approach to chronic disease screening could prove useful to inform clinical and therapeutic decision making in a high-risk subgroup of dyslipidemic patients. In addition, the benefits of APOE genotyping include the identification of South African patients with FH, which remains underdiagnosed and undertreated in general medical practice. This could allow clinicians to prioritize the need for lipid-lowering pharmacotherapy while also preventing statin overtreatment in patients expected to show a more favourable response to lifestyle-based interventions. A critical appraisal of the potential for APOE genotyping to add value to patient management beyond a limited diagnostic scope or as a routine component of cardiovascular risk screening could also prove useful in validating the appropriateness of genomic testing to not only inform clinical and therapeutic decision making as part of pre-symptomatic prevention of AD, but also to improve long-term health outcomes in the context of the metabolic syndrome and its associated comorbidities.

d) CONCLUSIONS

We conclude that, in order to advance the routine clinical implementation of a genomics-based preventive strategy aimed at combating the health burden imposed by the growing global prevalence of AD and dementia, an effective framework is required to support a continuum of research translation aimed at realizing the application of emerging genomic technologies to improve quality of life and promote wellness throughout life.

7. ACCUMULATED OUTPUTS

A set of standard operating procedures (SOPs) were developed for the SNPs analysed, which formed part of student training:

The results of the study will be presented at an international Winehealth Congress in Australia during 2013 as well as the Winehealth2015 congress in Tours, France during 2015.

The results were submitted for publication in a peer reviewed journal, as well as in the popular press (Wynboer, Tygerberg research report)

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

Establishment and ongoing development of a research database linked to the service component of a pathology-supported genetic testing service

The industry will benefit from the results of this study in the following areas:

- The comparison of the effect of moderate red wine consumption to brandy on the atherogenic lipoprotein profile, oxidative stress and inflammatory status has enabled us to document the effect of brandy in relation to the well documented health benefits of moderate wine consumption.

- The genetic screen of CVD risk factors identified the proportion of individuals who may not benefit from drinking alcohol as a consequence of gene-environment mismatches, based on the mutation/allele frequencies in the study population.

- With this information the presence of genetic risk factors can in future be correlated with relevant biochemical parameters to compare gene expression in the presence and absence of known environmental triggers.

- We were also be able to determine the impact of mutations/functional polymorphisms included in the genetic screen on the response to the intervention to ultimately develop guidelines for safe drinking habits.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

The health benefits of moderate alcohol consumption have been documented, and the added benefit of the anti-oxidants in red wine demonstrated that red wine have more health benefits than brandy. Individuals that may not benefit from alcohol intake can now been identified according to their genetic profile. This will enable medical professionals to advise their patients on the possible harm alcohol may have for certain individuals.

Industry may use this information to combat the anti-alcohol lobby from various governmental and non-governmental organizations victimizing all consumption of alcoholic beverages. Moderate alcohol consumption may have health benefits in certain individuals.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Student Name and Surname	Student Nationality	Degree (e.g. MSc Agric, MComm)	Level of studies in final year of project	Total cost to industry throughout the project
Honours students				

Masters Students				
Hlmar Luckhoff	South African	MSc	MSc	10000
Kobus Pretorius	South African	MSc, HonsBSc	MSc	10000
Leslie Fisher	South African	MSc	MSc	10000
PhD students				
Nicole van der Merwe	South African	PhD, HonsBSc	PhD	10000
Postdocs				
Support Personnel				

PERSONS PARTICIPATING IN THE PROJECT (Excluding students)

Initials & Surname	Highest Qualification	Degree/ Diploma registered for	Race (1)	Gender (2)	Institution & Department	Position (3)	Cost to Project R
Dr D P van Velden	M Phil		W	M	University of Stellenbosch	PI	20 000
Prof MJ Kotze	PhD		W	F	University of Stellenbosch	PL	40 000
Dr M Kidd	PhD		W	M	University of Stellenbosch	Co	1 000

(1)Race B = African, Coloured or Indian
W = White

(2)Gender F = Female
M = Male

(3)Position Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

1. Lückhoff HK, Brand T, van Velden DP, Kidd M, Fisher LR, van Rensburg SJ, Kotze MJ. Clinical relevance of apolipoprotein E genotyping based on a family history of Alzheimer's disease. Current Alzheimer Research 2015; 12(3):210-217.

2. van Velden DP, Kotze MJ, Blackhurst D, Kidd M. Health claims on the benefits of moderate alcohol consumption in relation to genetic profiles. Journal of Wine Research, 2011, Vol 22, pp. 123-129.
3. Van Velden DP. Gesondheidsvoordele van wyn is afhanklik van interaksie tussen gene en omgewing. Wynboer, Augustus 2010; 106-107.
4. Kotze MJ, van Velden DP. Waar staan ons nou met alkohol en gesondheid? Wynboer, Oktober 2011
5. van Velden DP van der Merwe S, Fourie E, Blackhurst DM, Kidd M, Kotze MJ, Mansvelt EPG. The influence of a Mediterranean-like diet with and without red wine on patients with the metabolic syndrome. S Afr J Enol Vitic. 2007;28 (1): 44-49.
6. Mansvelt EPG, Fourie E, Blackhurst D, Kotze T, Stofberg H, van der Merwe S. Kotze MJ, van Velden DP. The influence of a Mediterranean Diet with and without red wine on the haemostatic and inflammatory parameters of subjects with the metabolic syndrome. S Afr J Enol Vitic. 2007;28 (1): 37-43

e) PRESENTATIONS/PAPERS DELIVERED

- Lückhoff HK, van Rensburg SJ, Kotze MJ. A novel clinical application for apolipoprotein E genotyping: A critical window of opportunity to reduce the burden of late-onset Alzheimer's disease in the general population. SONA conference 2015

- Lückhoff, Kidd M, van Rensburg SJ, Kotze MJ. Benefits of Apolipoprotein E genotyping in dyslipidemic patients with a family history of Alzheimer's disease to optimize cardiovascular risk management. SASHG conference 2015

- Lückhoff HK, van Rensburg SJ, Kotze MJ. A novel clinical application for apolipoprotein E genotyping: A critical window of opportunity to reduce the burden of late-onset Alzheimer's disease in the general population. Pathology Research Day NHLS 2015

- 4th International Wine and Health Conference, 3-6 October 2010, Friuli, Italy. Invited speaker: Health claims on the benefits of moderate alcohol consumption in relation to genetic profiles

- Winehealth2015 Congress October 30 to November 1, 2015 in Tours, France. Invited speaker on: "Responsibility of media coverage and media attitudes towards Science and Technology"

Industry allocated project number	PHI allocated project number

8. BUDGET

TOTAL COST SUMMARY OF THE PROJECT

TOTAL FUNDING REQUIRED FOR FOLLOWING YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL

Overheads (only if part of project cost)					20000	10000		
Research Personnel (insert additional rows if needed)					40000	20000		
Research and Technical Assistance (directly linked to project) (insert additional rows if needed)					15 000	7500		
Bursaries								
Research materials and supplies -specify each item (insert additional rows if needed)								
DNA extraction kits					10 000	5000		
PCR mastermix, buffers					15 000	7500		
Primers / probes					10 000	5000		
Size ladder					3 000	1500		
Agarose, tips, tubes					12 000	6000		
Other (gloves, stationary, etc.					15 000	7500		
Research Equipment (insert additional rows if needed)								
Local travel and accommodation								
Local conferences (only specify separately for THRIP purposes)					20 000	10 000		
Capital items *								
Other								