CFPA	SAAPPA / SASPA / SAT	DFTS	Winetech
Canning Fruit Producers' Assoc. Submit to: Wiehahn Victor Tel: +27 (0)21 872 1501 inmaak@mweb.co.za	Fruitgro Science Submit to: Louise Liebenberg Tel: +27 (0)21 882 8470/1 louise@fruitgro.co.za	Dried Fruit Technical Services Submit to: Dappie Smit Tel: +27 (0)21 870 2900 dappies@dtd.co.za	Submit to: Anel Andrag Tel: +27 (0)21 807 3387 andraga@winetech.co.za

			X

Indicate (X) client(s) to whom this final report is submitted.
Replace any of these with other relevant clients if required.

FINAL REPORT

FOR [Click HERE and type year]

PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Prof MJ Kotze	
Present position	Senior lecturer and Researcher	
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PROJECT INFORMATION

Project number	N09/08/224
Project title	Development and application of a pathology supported genetic assay to assess the impact of hereditary factors on health outcomes in individuals subjected to a wellness screen
Project Keywords	Alcohol, genetics, metabolic syndrome, pathology

Industry programme	**CFPA**	
	Deciduous	
	DFTS	
	Winetech	x
	Other	

Fruit kind(s)	grapes
Start date (dd/mm/yyyy)	1 March 2010
End date (dd/mm/yyyy)	30 February 2012

FINAL REPORT

(Completion of points 1-5 is compulsory)

1. Executive summary

Give an executive summary of the *total* project in no more than 250 words

The purpose of the project was to assess the impact of hereditary factors on biochemical parameters of cardiovascular risk in relation to moderate alcohol consumption

We focused on patients with features of the metabolic syndrome (MetS) characterised by three or more of the following features: Central obesity, hypertension, glucose intolerance and dyslipidaemia (Low HDL-cholesterol and /or high triglyceride levels). MetS is associated with an increased risk of many chronic diseases including cardiovascular disease (CVD) and Alzheimer's disease (AD). Realisation that the E4 allele of the apolipoprotein E (ApoE) gene provides a genetic link between CVD and AD highlighted the importance of addressing shared disease mechanisms before or early in disease development in order optimize health in later life (Kotze and van Rensburg 2012). A questionnaire was used to denote clinical and lifestyle information in more than 400 study participants. Details on alcohol consumption and weekly food intake over the last 3 months at assessment was obtained, as well as information on family history, own medical history, health concerns, medication use, and lifestyle risk factors.

As a result of this study and an overlapping alcohol intervention study led by Dr DP van Velden, the well-established protective effect of moderate alcohol consumption on CVD risk factors has been confirmed in the local population. Since the genetic profile influences the effect of alcohol on biochemical parameters of CVD risk, safe limits of wine and brandy consumption may in future be based partly on the genetic profile or knowledge of the relevance of gene-environment interaction.

2. Problem identification and objectives

State the problem being addressed and the ultimate aim of the project.

While alcohol has been classified as a functional food it is also considered a drug when abused. Research relating to gene-diet (nutrigenetics) and gene-drug (pharmacogenetics) interactions in relation to moderate alcohol intake is therefore warranted. Every disease susceptibility or medical condition is caused, regulated or influenced by genes.

Although the antioxidant effects of red wine is well documented, alcohol intake may be contra-indicated in individuals with certain genetic alterations that occur relatively frequently in the general population. For example, alcohol interacts with the apolipoprotein E (ApoE) E4 gene variant to increase the risk of Alzheimer's disease, especially when other CVD risk factors such as hypertension or obesity are also present. Cancer risk may also be increased with high alcohol intake, particularly when folate status is low in mutation carriers of methyltetrahydrofolate reductase (MTHFR) gene. Alcohol furthermore increases the absorption of iron that is of specific concern in HFE gene mutation carriers with non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome.

As with clinical pathology and nutrition, genomics is relevant to every medical speciality. Long-term intervention strategies for health promotion including lifestyle modification and safer daily drinking habits may therefore be more effective when guided from the genetic background. This does not only apply to alcohol intake but also drinks that contain certain substances or stimulants such as caffeine (Cornelis et al. 2006; Palatini et al. 2009). A major objective of this project was to develop cost-effective laboratory assays that can facilitate disease prevention, diagnosis and treatment.

Project number no N09/08/224, Prof MJ Kotze, University of Stellenbosch

The specific aims of the study include the following:

1. Development of a cost-effective multi-gene test to be used in a wellness program
2. Development of a gene-based Wellness Program to reduce the risk of cancer and heart disease in patients with (symptoms of) the metabolic syndrome.
3. Genotype-phenotype correlation studies to identify possible statistically significant associations.

Development of a unique pathology supported genetic testing (PSGT) approach - that involves the evaluation of gene expression as reflected by biochemical abnormalities / pathology that in turn could be used to monitor response to treatment - provides a solid basis for application of personalised medicine. The next step is full implementation of genomic medicine, with a special focus on the proven beneficial effects of light to moderate alcohol intake on inflammation as a key disease mechanism across diagnostic boundaries.

3. Workplan (materials & methods)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage and examination stages and parameters.

Descriptive and product development study

A questionnaire was used to document personal details, clinical characteristics, potential environmental risk factors (e.g. smoking, excessive alcohol intake, nutrient deficiencies) and family history of the study participants. Clinical assessments included blood pressure, waist and hip circumference and/or body mass index (BMI). Use of chronic medication and food supplements were documented together with any food allergies or intolerances reported in order to identify potential gene-drug (pharmacogenetics) or gene-diet (nutrigenetics) interactions relevant to the genes tested.

Biochemical determinations included a fasting lipid profile, homocysteine levels, C-reactive protein / hs-CRP, iron status, insulin, glucose levels, liver function tests, as well as measurement of insulin resistance, liver function, vitamin D and anti-oxidant markers of lipid peroxidation where possible, using standard methods.

Genetic studies were performed after obtaining informed consent for genetic studies. Genetic risk factors were be analysed in conjunction with relevant environmental risk factors and biochemical parameters. The gene mutations / functional polymorphisms analysed have previously been described in the context of CVD risk (Kotze et al. 2003; Kotze and Thiart 2003) and provided the prototype for the current study. More functional genetic variants were added relevant to various common chronic disorders, following an extensive literature search in the field. Genetic testing was be performed using polymerase chain reaction (PCR)-based methods (e.g. direct sequencing, real-time PCR) and other advanced testing methods and devices aimed at cost reduction in the near future.

4. Results and discussion

State results obtained and list any benefits to the industry. Include a short discussion if applicable to your results. This final discussion must cover ALL accumulated results from the start of the project, but please limit it to *essential* information.

More than 400 study participants were enrolled in the study after providing informed consent. Genotyping was performed using real-time polymerase chain reaction (RT-PCR) TaqMan technology analytically validated against direct sequencing as the gold standard. A multi-gene assay was optimised for genotyping of clinically-useful single nucleotide polymorphisms (SNPs) in the ApoE, MTHFR, Factor II (prothrombin), Factor V, HFE and CYP2D6 genes

(2010). The SNP panel was extended to include functional genetic variations in the FTO, PPARg, TNFa, GSTT1, GSTM1, MnSOD and COMT genes (2011), as well as the PAI-1, CYP2D6, FABP2 and ADRB2 genes (2012).

In 2012 ethics approval was obtained to select control samples from our online database in a comparative study of patients with non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome. The effect of the tumor necrosis factor-alpha (TNF-a) -308G>A (rs1800629) SNP on inflammation and disease progression was studied in 119 patients with fatty liver identified on ultrasound, including 88 histologically confirmed NAFLD patients, and 166 control individuals. Liver function tests, determination of insulin resistance and C-reactive protein (CRP) levels were performed using standard techniques. The minor allele frequency of TNF-a -308G>A was significantly higher in the total NAFLD (p=0.047) as well as NASH subgroup (p=0.030) compared with obese patients without a histologically confirmed NAFLD diagnosis. The onset of fatty liver disease symptoms was on average 5 years younger in the presence of each risk-associated TNF-a -308G>A A-allele (p=0.028). A significant association was furthermore observed between the number of TNF-a -308G>A minor alleles and increasing C-reactive protein levels (p=0.029), with a favourable reduced effect in the presence of low to moderate alcohol intake. Patients with alcohol intake (below the cut-off level for diagnosis of NAFLD) had on average 70% reduced CRP levels compared with patients who abstained from alcohol drinking (p=0.038). Identification of the TNF-a -308G>A SNP as a genetic determinant of NAFLD severity is in accordance with previous mRNA expression studies performed in South African patients with this condition. As the gene effect depends on the level of expression that may be reflected by raised CRP levels in response to alcohol consumption, TNF-a -308G>A genotyping should routinely be combined with relevant blood biochemistry levels to determine current or future clinical relevance.

Milestone	**Achievement**
1. Complete health questionnaire and assay development	The study questionnaire has been developed in relation to the genes studied. The diagnostic and risk reduction cardiovascular disease (CVD) multi-gene test was optimized on a cost-effective real-time PCR mutation detection platform (previously strip-assays). Positive and negative controls were identified for each of the SNPs analysed and used to validate the accurateness of the TaqMan assays against direct sequencing as the gold standard for mutation detection. Additional SNPs are added on an ongoing basis as part of student projects contributing to the development of the SNP Knowledge Database and online genetic database. This provides a valuable resource for selection of cases and controls in an increasing number of research projects making use of data mining using our statistical tool.
2. Clinical assessment and sample collection for implementation of individualised intervention plans.	Reports have been prepared by using the Gknowmix Software for data integration and interpretation for more than 300 study participants. These include reports provided to patients referred by 17 medical doctors who registered for the Integrative Medicine distance learning course of the University of Stellenbosch, of which most afterwards sent us a blood sample of themselves and/or a spouse for the same genetic test, another 20 reports to a registered dietician who incorporated the CVD multi-gene test as part of the Wellness Program of a medical scheme in Namibia, 27 patients diagnosed with breast cancer, 37 healthy volunteers who participated in an alcohol intervention study, and 50+ patients referred by a psychiatrist who was interested to determine the

	impact of genetic variation in the MTHFR and raised homocysteine levels in patients with depression. High homocysteine levels reflect a disturbance in DNA methylation known to be associated with depression and the metabolic syndrome. This doctor is in the process to write an article on the clinical usefulness of the test she used in clinical practice. Alcohol, smoking and folate intake influence homocysteine levels and are taken into account during generation of patient reports signed off by qualified medical scientists or medical technologists registered at the HPCSA.
3. Complete genetic analysis and generate a test report for each patient.	Based on the results of a questionnaire-based survey performed among healthcare practitioners in 2010, feedback is provided as follows to study participants in a comprehensive report: 1) PATHOLOGY SUPPORTED GENETIC TEST RESULTS: Table with genetic test results in the context of the family history, current health status (e.g. biochemistry blood levels), use of medication and lifestyle information (e.g. activity level and diet scores) 2) GENETIC COUNSELLING SUPPORT: Detailed description of the importance and implications of genetic variations detected in the genes included in the analysis 3) DNA-LIFESTYLE RISK REDUCTION PLAN: This part of the report serves as a handout to study participants on how to adjust their diet and lifestyle based partly on the genetic test results obtained, integrated with pathology/biochemical measurements to determine gene expression and response to treatment. It is made clear that this program is considered experimental at present, therefore its effectiveness must be evaluated as part of a long-term health outcome research project. Cheek swab samples were obtained from 25 individuals who attended the Wellness Day at Tygerberg Hospital and a process developed for return of results to about half of these individuals who expressed an interest to receive a test report during a post-test counselling/consultation session.
4. Post-test consultation and implementation of individualised diet and lifestyle intervention programs.	The Genomic Health and Wellness Program developed for patients with features of the metabolic syndrome will be implemented during 2013. A pathology supported genetic testing (PSGT) service will be applied that combines relevant clinical, pathology/biochemistry and genetic information for clinical decision-making (Kotze and van Rensburg 2012). Although evidence-based guidelines should ideally be developed before implementation of genomic applications, Khoury et al. (2007) acknowledged the need to fill the remaining information gaps through ongoing data collection and health outcome studies. PSGT involves a combined service and research approach that seeks to evaluate “real world” health outcomes of genomic applications, whereby overlapping aspects of translation research could provide feedback loops to allow integration of new genetic knowledge into clinical care as suggested by Khoury et al. (2007). The strategy defined by these authors to accelerate the appropriate incorporation of genomics into clinical practice, which will be applied during the implementation phase of this study approved for THRIP funding from 2013.

The well-established protective effect of moderate alcohol consumption on cardiovascular disease (CVD) risk factors has been confirmed. Since the genetic profile influences the effect of alcohol on biochemical parameters of CVD risk, safe limits of alcohol consumption may in

future be based partly on the genetic profile or knowledge of the importance of genetic variation in this context.

5. Accumulated outputs

List ALL the outputs from the start of the project.
The year of each output must also be indicated.

Technology development, products and patents

Indicate the commercial potential of this project (intellectual property rights or a commercial product(s)).

This study enabled determination of the potential benefits of including genetic testing as part of wellness programs. Based on this and the results obtained from an ethically approved survey questionnaire distributed to more than 2000 SA healthcare practitioners during 2010, a framework has been established for incorporation of genetic testing in daily clinical practice. The 7th Applied Genetics Workshop on the 26th of October 2012 was very successful with nearly 100 healthcare professionals attending.

Human resources development/training

Indicate the number and level (e.g. MSc, PhD, post doc) of students/support personnel that were trained as well as their cost to industry through this project. Add in more lines if necessary.

	Student level (BSc, MSc, PhD, Post doc)	Cost to project (R)
1.	8 BSc (Hons)	R 160 000
2.	3 MSc	R 120 000
3.		
4.		
5.		

Publications (popular, press releases, semi-scientific, scientific)

Van Rensburg SJ, Potocnik FCV, Kotze MJ and Stein DJ. Antemortem markers. In: Principles and Practice of Geriatric Psychiatry Third Edition. Ed. MT Abou-Saleh, CLE Katona and A Kumar. Johan Wiley, UK, 2010, pp299-303 (ISBN: 978-0-470-74723-0).

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; 22: 123-129.

Kotze MJ, van Velden DP. Waar staan ons nou met alkohol en gesondheid? WynLand, Oktober 2011.

Davis W, de Kock L, Pretorius K, Schoeman R, van Rensburg SJ, Kotze MJ. Development of a single nucleotide polymorphism (SNP) knowledge database: ASMT rs4446909, rs598968 and MTHFR rs1801133 as susceptibility alleles for depression. African Journal of Psychiatry Sept 2011: 5.

Van Rensburg SJ, Kotze MJ, Potocnik FCV, Hon G, de Klerk M, Erasmus RT. Leukcocyte counts in multiple sclerosis and Alzheimer's disease. African Journal of Psychiatry Sept 2011: 10.

Kotze MJ, van Velden DP, Kidd M, Marnewick J. Genotype associations in South African patients with the metabolic syndrome. African Journal of Psychiatry Sept 2011: 11.

Kotze MJ and van Rensburg SJ. Pathology supported genetic testing and treatment of cardiovascular disease in middle age for prevention of Alzheimer's disease. Metab Brain Dis 2012; 27: 255-266.

Presentations/papers delivered

The research results were presented at the following congresses"

Maart 2011: Human Genetics Congress, Cape Town

August 2011 & 2012: Academic Yearday, Stellenbosch University

September 2011: Biological Psychiatry Congress, Cape Town

Septmber 2012: Nutrition Congress, Bloemfontein

The following abstract was accepted for a oral presentation at the 2013 Wine Health Congress to be held in Sydney, Australia, 16-21 July:

Kotze MJ, Marnewick JL, Kidd M and van Velden DP. Assessment of the impact of hereditary factors on biochemical parameters of cardiovascular risk in relation to moderate alcohol consumption.

4. Total cost summary of project

	Year	CFPA	Deciduous	DFTS	Winetech	THRIP	Other	TOTAL
Total cost in real terms for year 1					R 110 000	R 55 000		**R 165 000**
Total cost in real terms for year 2					R 120 000	R 60 000		**R 180 000**
Total cost in real terms for year 3					R 130 000	R 65 000		**R 195 000**
Total cost in real terms for year 4								
Total cost in real terms for year 5								
TOTAL					**R 360 000**	**R 180 000**		