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عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	تعدد الأشكال الجينية لجينات APOE و CLU وخطر الإصابة بمرض الزهايمر في المجتمع السعودية
Title of the proposed project in English	Association of APOE and CLU gene polymorphisms with the risk of Alzheimer's disease in Saudi subjects
المشرف الرئيس - PI	Amani Alghamedi
التخصص الدقيق للمشرف الرئيس - Specialty of PI	Neurobiochemistry
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المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	12 months
Abstract of the proposal (No more than 200 words)	Alzheimer's disease (AD), is the most common form of dementia and approximately one in eight people over 65 years old are at risk worldwide. It is a degenerative brain disease characterized by the development of amyloid plaques and neurofibrillary, or tau, tangles; the loss of connections between nerve cells in the brain; and the death of these nerve cells. The intricacies of the

	mechanism of AD have not yet been clearly defined. The risk factors for AD are age, female gender, diabetes mellitus, hypertension and cerebrovascular diseases. Among the Saudi population the risk factors thought to be associated with the onset and pathogenesis of AD are not exactly identified. Genome-wide associated studies (GWAS) have identified lipid metabolism, inflammatory response and endocytosis as the three main pathways involved in AD risk. Thus, we propose to validate various blood biochemical markers of AD including , Aβ-metabolism related proteins (including Aβ1–42 , Aβ1–40), biomarkers for Lipoprotein metabolism (Total cholesterol, LDL and Lipoprotein A), and clusterin which could bind amyloid-beta (A beta) peptides and prevent fibril formation. Furthermore we intend to investigate the genetic polymorphisms in genes belonging to cholesterol Metabolism ,Apolipoprotein E (ApoE) and clusterin (CLU), in relation to AD and its measured biochemical markers in samples from Saudi Arabia.
Hypothesis of the proposal	Influence of the Single nucleotide polymorphisms of APOE and CLU gene on risk of AD in Saudi population
Specific objectives	1. To investigate the relationship between established biochemical markers of AD including Aβ-related proteins ,Aβ1–42 and Aβ1–40 and outcome of the disease. 2. To investigate the relationship of the serum levels biomarkers for Lipoprotein metabolism (Total cholesterol, LDL ,Lipoprotein A and clusterin) with outcome of the disease. 3. To determine the association between genetic variants in APOE (rs7412, rs429358)and CLU (rs1113600, rs1532278) and biochemical parameters (measured in objective 1 and 2 above) and its association with the occurrence of AD in samples from Saudi ethnic population.
Methodology & Major Techniques to be used	1. Collect detailed information from the patients and age matched control subjects with a particular focus on the family history of AD/Demintia, medications, diet, chronic disorders, and lifestyle habits Ethical approval will be obtained from the Ethics Committee of the Research Center, College of Science, King Saud University prior to the study. Written consent will also be obtained from the subjects prior to their inclusion in the study. Saudi subjects between the ages 65-90 years (N =100) with a confirmed diagnosis of AD and no treatment of any kind and an equal number of age matched controls will be recruited for the study. Subjects with malignancy, endocrine, cardiac or lung disease will be excluded from the study.The subjects

will be recruited from King Khalid Hospital ,Riyadh.with the help and coordination of physicians with proven expertise in the field.

AD will be diagnosed in the subjects following the NINCDS-ADRDA and the DSM-IV criteria
A generalized questionnaire seeking the details of present and previous medications dietary habits, chronic disorders, lifestyle activities including cigarette smoking, and exercise and a family history of AD will be obtained from the subjects. Subjects with a history of Dementia or AD treatment and those currently receiving any medications such as steroids that are known to interfere with metabolism and patients with documented disease, and malignancies and will be excluded from the study. Clinical and anthropometric parameters will be collected from both the patients and the controls by trained and experienced personnel. Subjects with normal NINCDS-ADRDA and the DSM-IV scores that are free of any metabolic bone diseases based on the medical history provided in the structured questionnaire will be considered as controls.
The anthropometry included height, weight, waist and hip circumference utilizing a standardized measuring tape in cm; systolic and diastolic blood pressure measurements; and body mass index(BMI) (calculated as kg/m2).
Blood will be collected in EDTA tube for genomic DNA analysis. For collection of serum, blood will be transferred immediately to plain a non-heparinised tube and kept undisturbed for some time at room temperature to allow clotting, the clot will be removed by centrifugation. The resulting serum will be transferred to pre-labelled plain tubes and stored at -20°Cuntil analysis
2. Measuring the levels of the biochemical markers of Alzheimer disease including Aβ-related proteinsAβ1–42 and Aβ 1–40in subjects as well as in age matched controls.
Enzyme-linked immunosorbent assay (ELISA) will be used to measure plasma concentrations of both these markers, according to manufacturers instructions.

3. Measuring the levels clusterin ,Total cholesterol, LDL and Lipoprotein A in patients and age matched controls
Plasma concentration of Clusterin will be measured using the ELISA technique according to the manufacturer's instructions. The inter and intra-assay coefficient variations will be verified. Measure Total cholesterol, LDL and Lipoprotein A in the serum of patients and age matched controls will be measured using autoanalyzer.

4. Analysis of polymorphisms in the APO E and CLU, genes.
High quality genomic DNA will purified from the whole blood by using the All Prep DNA mini kit according to manufacturer’s instructions and will be quantified spectrophotometrically. Two SNPs in APOE (rs7412, rs429358)and two in CLU (rs1113600, rs1532278) gene will be evaluated using allelic discrimination Real-time PCR using pre-designed TaqMan ® Probes from

	Applied Bio-systems, Foster City, CA, USA.
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	In the process
Recent References	1. .Shankarappa B.M., Kota L.N., Purushottam M., Nagpal K., Mukherjee O., Viswanath B., Varghese M., Jain S. Effect of CLU and PICALM polymorphisms on AD risk: A study from south India (2017) Asian Journal of Psychiatry, 27 , pp. 7-11. 2. FaranakMohammadpourLashkari, Anahita MohseniMeybodi, Zahra Mansouri, Hamid Kalantari, KameliaFarahmand, HamidrezaVaziri.The association between (8390G>A) single nucleotide polymorphism in APOE gene with Alzheimer's and Parkinson disease. The Egyptian Journal of Medical Human Genetics (2016) 17, 185–189 3. Al-KhedhairyAA : Apolipoprotein E polymorphism as a predictor for cognitive decline and dementia in Saudi general population over 65 years old.Genetics and Molecular Biology, 2004;27(3): 331-334. 4. Weinstein G, Beiser AS, Preis SR, et al. Plasma clusterin levels and risk of dementia, Alzheimer's disease, and stroke. Alzheimer's & Dementia : Diagnosis, Assessment & Disease Monitoring. 2016;3:103-109. doi:10.1016/j.dadm.2016.06.005. 5. Mayeux R, Schupf N. Blood-based biomarkers for Alzheimer's Disease: Plasma Aβ40 and Aβ42, and Genetic Variants. Neurobiology of aging. 2011;32(Suppl 1):S10-S19