21)

عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	تأثير طفرة نقطية في جين CYP1B1 على العصب البصري الذي قد يسبب الغلوكوما
Title of the proposed project in English	The Effect of point mutation in CYP1B1 gene on optic nerve which may cause Glaucoma
المشرف الرئيس - PI	د.أماني الغامدي
التخصص الدقيق للمشرف الرئيس - Specialty of PI	الكيمياء الحيوية للاعصاب
المشرف المساعد - Co-PI	Dr. Bahauddeen M Alrfaei
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	12 شهر
Abstract of the proposal (No more than 200 words)	Glaucoma is a leading cause of blindness. It is divided into two major types: Primary Open Angle Glaucoma and Primary Congenital Glaucoma (PCG). The prevalence of PCG varies geographically, from a rate of 1:10000 in Western countries to 1:1250 in the Romany population of Slovakia [1]. PCG is caused by the most common mutation, CYP1B1 [1], which has been confirmed in the

	western region of Saudi Arabia [2]. Since no data that cover all regions of Saudi Arabia are available, we predicted that mutation in CYP1B1 is still the most common mutation linked to PCG in Saudi Arabia based on the available studies. PCG variant p.Gly61Glu has been identified as the major disease-associated mutation in Saudi Arabia [2]. It is not known how CYP1B1 p.Gly61Glu damages the optic nerve or leads to blindness during development. The molecular aspects and consequences of having CYP1B1 p.Gly61Glu variant are not understood. We would like to study the effects of CYP1B1 mutation on the supporting cellular components of the optic nerve. We will examine how protein phosphorylation might be altered in retinal ganglion cells, oligodendrocyte function. In addition, we will assess myelin production, and the expression level of cytokines relevant to the mutation
Hypothesis of the proposal	The hypothesis of the study that CYP1B1 p.Gly61Glu damages the optic nerve by affecting different biochemical pathway such as phosphorylation pathways, cytokines production and expression levels of melatonin
Specific objectives	• Does the CYP1B1 mutation have an effect on phosphorylation pathways? • Does point mutation such as one amino acid substitution in CYP1B1 changes cytokines production? • Does the CYP1B1 mutation have an effect the expression levels of melatonin
Methodology & Major Techniques to be used	Phosphorylation: We will grow all cell lines in the lab using standard cell expansion methods and then compare the wild type to the cell lines possessing the mutation. We will purchase a phosphorylation detection kits to estimate if phosphorylation or certain pathways play a role in the deficit. It is unknown whether the CYP1B1 p.Gly61Glu mutation disturbs ganglion cells or supporting cells, such as glial cells and schwann cells. We will harvest cultured cells and collect protein lysate as previously referenced (10). We will then consume a Proteome Profiler Human Phospho-Kinase Array Kit, which can be purchased. This method is equivalent to ELISA. Any type of phosphorylation will be measured. The differential expression will be validated with western blots. The array method has been published previously (11). To consider any limitation in our research we might face no detection to any differences in phosphorylation. This result might conclude that the CYP1B1 mutation does not have a straight influence on glial cells, and Schwann cells. Our conclusion should be that the illness development has an exterior motivation that is discrete from phosphorylation pathways. Subsequently,

	toxicity experiments will inform us more about cell survival and viability. Cytokines Cytokines and chemokines provide suggestions about the situation around the optic nerve. We will measure the cytokines and chemokines excreted by glial cells and Schwann cells. We will compare the wild type to the p.Gly61Glu mutated cell lines, and we will buy commercially available panels to assess the level of cytokines and chemokines. For instance, an enzyme-linked immunosorbent assay (ELISA) (12) can be used. We will collect culture media after 24, 36 and 48 hours of culture. Then, we will use the gathered media in the ELISA method. This method will detect defined cytokines linked with glial cellsn cells (e.g. microglia) and Schwann cells. Independent discrete commercial kits will be used based on cell type. The kit should have cytokines distinct for that certain cell line. Negative results articulate that cytokine environments have no obvious link to glaucoma degeneration. The expression levels of melatonin We will evaluate the expression levels of melatonin and of retinol using the immunoblotting method as published (21).
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Not needed
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