18)

عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	المسح الجيني لطفرات جين KCNJ11 المسببة لمرض السكري ، كأساس لاستراتيجات الوقاية من المسببات الجينية لمرض السكري في السعودية.
Title of the proposed project in English	Genetic screening of neonates for mutations in KCNJ11 gene causing diabetes, as a basis for strategies to prevent genetic diabetes in Saudi population.
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التخصص الدقيق للمشرف الرئيس - Specialty of PI	Clinical Biochemistry
المشرف المساعد - Co-PI	Dr. Ihtisham Bukhari
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)	Neonatal diabetes occurs in new born in age of six months, it has genetic and non-genetic factors. Neonatal genetic diabetes is monogenic which is rare type of diabetes caused by pancreatic b-cells dysfunction. The mutations in the KCNJ11 gene are the most common cause of neonatal diabetes which is typically diagnosed in the first six months of life. The pathogenic mutations in

	this gene have been extensively studied in neonates or infants less than age of 6 months. Literature survey revealed that postnatal genetic screening of Saudi neonates and infants has never been studied previously. Therefore, current study is designed to investigate the pathogenic mutations and polymorphisms in Saudi neonates and infants. For current study blood samples from infants with age less than 6 months and new born babes with postnatal age of 3 days will be collected. Selected mutations in collected samples will be studied by SNP Genotyping and sanger sequencing. The current study will enable us to evaluate the genetic causes of neonatal diabetes and frequency of genetic mutations in Saudi neonates.
Hypothesis of the proposal	• This study will help to find frequency of certain mutations in diabetes associated genes in Riyadh, KSA. • This study will help to screen out the carrier individuals that will help to develop prenatal diagnosis and genetic counseling strategies to prevent genetic diabetes in Saudi population. • Moreover, through seeking pre implantation genetic diagnosis (PGD), families with known mutations may have the possibility of having children free from those mutations. • An identification of the KCNJ11 mutations has important therapeutic implications for patients; mutations carriers can switch from insulin injections to oral sulfonylurea, which results in improved glycemic control. • This study will help to establish the basis for the nationwide genetic diagnosis program for neonatal diabetes.
Specific objectives	• To identify the mutations in KCNJ11 gene causing neonatal diabetes in Saudi population. • To investigate the frequency of KCNJ11 mutations and association with other factors in Saudi neonates and infants. • Genetic counselling of the parents for prevention and control of the genetic diabetes.
Methodology & Major Techniques to be used	Blood Sampling: Blood samples from umbilical cord will be collected in EDTA tubes from the neonates at the time of birth or 3-4 drops of blood will be collected by heel puncture at sterilized filter papers. For some cases (age less than 6 months) 0.5-1cc venous blood in EDTA tubes may also be collected. Sampling will be performed at different hospitals of the Riyadh. After collection samples will be stored in -20 ^{o}C before DNA extraction.

	DNA extraction: Genomic DNA will be extracted by using Qiagen Genomic Extraction kits, which simply employ spin columns, for the isolation of DNA. The spin columns contain a silica resin that selectively binds DNA, depending on the salt conditions and other factors influenced by the extraction method. Genetic screening: Pathogenic mutations/polymorphisms neonatal diabetes associated in KCNJ11 gene will be investigated by SNP Genotyping Analysis Using TaqMan Assays. Special fluorescent probed primers will be designed and amplified by realtime PCR (Applied Biosystems, Foster City, CA). For results validation and confirmation, the identified mutations/polymorphisms will further be analyzed by Sanger sequencing methods. For this purpose, special primers will be designed for each of the targeted mutations, which will be amplified by conventional thermocycler (Applied Biosystems, Foster City, CA). The amplicons will be precipitated with ethanol and sequenced with Big Dye Terminator version 3.1 (Applied Biosystems, Foster City, CA). Sequenced samples will be electrophoresed on an ABI 3730 genetic analyzer, and the traces will be inspected by SeqMan software (DNAstar Lasergene version 5.0.221.0).
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Ethical approval is available
Recent References	• Ellard S, Flanagan SE, Girard CA et al (2007) Permanent neonatal diabetes caused by dominant, recessive, or compound heterozygous SUR1 mutations with opposite functional effects. Diabetes 53:2713–2718. • Fendler W, Pietrzak I, Brereton MF et al (2013) Switching to sulphonylureas in

children with iDEND syndrome caused by KCNJ11 mutations results in improved cerebellar perfusion. Diabetes Care 36:2311–2316.
• Garin I, Edghill EL, Akerman I et al (2010) Recessive mutations in the INS gene result in neonatal diabetes through reduced insulin biosynthesis. Proc Natl Acad Sci USA 107:3105–3110.
• Gloyn AL, Pearson ER, Antcliff JF et al (2004) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 350:1838–1849.
• Mlynarski W, Tarasov AI, Gach A et al (2007) Sulfonylurea improves CNS function in a case of intermediate DEND syndrome caused by a mutation in KCNJ11. Nat Clin Pract Neurol 3:640–645
• Sagen JV, Raeder H, Hathout E et al (2004) Permanent neonatal diabetes due to mutations in KCNJ11 encoding Kir6.2—patient characteristics and initial response to sulfonylurea therapy. Am J Hum Genet 81:375–382.