Supplementary Data For:

Discovery of a Genetic Metabolic Cause for Mauriac Syndrome in Type 1 Diabetes

Michael J. MacDonald[1]*, Noaman M. Hasan[1], Israr-ul H. Ansari[1], Melissa J. Longacre[1], Mindy A. Kendrick[1], Scott W. Stoker[1]

[1]Childrens Diabetes Center, University of Wisconsin School of Medicine and Public Health, Madison, WI 53706

*Correspondence to: Michael J. MacDonald, Email: *mjmacdon@wisc.edu*

TABLE OF CONTENTS

SUPPLEMENTARY DATA

CASE HISTORY

The patient was diagnosed with type 1 diabetes when he presented with a blood glucose level of 603 mg/dl, a serum bicarbonate level of 16 meq/l end an anion gap of 29 meq/l at age 30 months. Family history was pertinent in that his father was diagnosed with type 1 diabetes at age 6 years and died at age 45 years from complications of diabetes. Although his father's diabetes was very poorly controlled, the father never had an enlarged liver or growth failure. The patient's mother does not have diabetes (His mother always reported normal fasting blood glucose values when queried.) or an enlarged liver. At diagnosis the patient showed increased serum islet cell antibodies (80 JDF units (normal < 5 JDF units)) consistent with a diagnosis of type I diabetes. The patient has high risk type 1 diabetes HLA DR HLA DQ alleles. His HLADR type is DR4 DQ3 and since both parents are DR4 DQ3 positive, it is likely the patient possesses two copies of the DR4 DQ3 haplotype. At ages 7 1/12, 7 4/12, 7 10/12, 8 1/12, 9 9/12, 11 4/12, 11 8/12, 12 3/12 and 12 8/12, his hemoglobin A1c values were 7.9%, 8.8%, 6.8%, 8.3%, 9.1%, 9.2%, 8.8%, 10.7% and 10.2%, respectively (Normal hemoglobin A1c is < 6.0%. The target hemoglobin A1c for patients with type 1 diabetes is < 7.0%. A hemoglobin A1c higher than 9% reflects very poor glycemic control for the previous 2-3 months. His stated doses of insulin ranged from 1.7 to 2 units of insulin/kg/day which are higher than typical doses for a male child his age using conventional multiple daily insulin injection therapy (1). However, the patient admitted to frequently giving lower than the prescribed insulin doses to himself. At age 13 0/12 the patient was hospitalized for respiratory distress caused by the extremely enlarged liver interfering with diaphragmatic excursions. His liver extended down into his pelvis. His hemoglobin A1c value was 10.2% consistent with an average blood glucose value of 246 mg glucose/dl (14 mM glucose) (according to the National Glycohemoglobin Standardization Program conversion formula for hemoglobin A1c values measured by HPLC) or a higher average blood glucose value (as almost all of the hemoglobin A1c values were measured with the DCA 2000 Seimens/Bayer Analyzer on a finger-stick sample of blood in an outpatient setting which in our clinic underestimates the hemoglobin A1c value compared to the HPLC method. See below.) over the previous two to three months. At that time he was also neutropenic (blood neutrophil count = 510 cells/µl blood (normal = 1700-7500 neutrophils/µl blood)) (white blood cell count = 4000 cells/µl (normal = 4000-10,500 cells/µl blood)). The red blood cell count, the platelet count and the counts of other types of white blood cells were normal. Liver transaminase values in his blood were increased. The alanine aminotransferase level was 897 U/L and the aspartate aminotransferase level was 911 U/L (normal values = 16-63 U/L and 0-50 U/L serum, respectively). The serum albumin and total protein levels were 3.4 g/dl (normal values = 3.5-5.0 g/dl) and 6.4 g/dl (normal values = 6.4-8.3 g/dl). A liver biopsy showed cells swollen with glycogen and no inflammation and no steatosis. An abdominal ultrasound examination was consistent with homogeneous glycogen deposition throughout an extremely enlarged liver (23 cm in its greatest (craniocaudal) dimension). He showed no signs of puberty. The neutropenia improved during the hospitalization. Three months after the hospital admission, the size of his liver decreased to 4 cm below his right costal margin, the neutropenia had disappeared, blood transaminase enzyme values were normal and the hemoglobin A1c had improved to 8.8%.

At ages 13 9/12 to 14 7/12 the patient was seen as an outpatient for diabetes care. At these times his liver had again increased in size and was palpable down to below his umbilicus and his hemoglobin A1c values ranged from 9.2% to 10.0% during this time. At age 15 6/12 the hepatomegaly worsened and the neutropenia recurred. His blood neutrophil count at this time was 600 neutrophils/µl blood and his white blood cell count was 2800 cells/µl blood. His blood ceruloplasmin level was normal and blood hepatitis A and hepatitis C titers were nonreactive.

SUPPLEMENTARY DATA

At age 15 4/12 years, the patient's skeletal age was 14 years which was within one standard deviation from his age and was normal. According to his mother, his height was average from birth until age 6 years. At age 7 3/12 his height was in the 25th percentile for his age. At age 12 3/12 his height was in the 5th percentile for his age and subsequently it decreased to the second percentile at 13 0/12. Since then his height percentile steadily decreased until at age 15 6/12 it was in the 0.09 percentile for his age.

Throughout the course of the patient's diabetes he frequently mentioned that small increases in his dose of insulin caused disproportionately large decreases in his blood glucose levels. He believed he was abnormally susceptible to hypoglycemia. These claims seemed to borne out by the patient's medical history after his compliance improved as the low blood glucose levels were documented with fingerstick blood glucose testing and the low blood glucose level could not be explained by other factors, such as eating less than usual amounts of food or exercising more than usual. The patient was very sedentary and did not exercise. His fear of using insulin to maintain his blood glucose close to recommended target levels caused the hyperglycemia. Although many diabetes patients make similar claims, judging from details of his medical history, in this patient's case the perception of unusual susceptibility to hypoglycemia might have been accurate.

Blood glucose testing by the patient: The patient supplied almost no records of blood glucose values obtained with a personal blood glucose meter between the ages of about 10 years and 17 years. At ages 17 3/12 and 17 8/12 he did supply blood glucose records that might confirm his impression that he possessed an increased sensitivity to the hypoglycemic action of insulin. For example, a blood glucose of 407 mg glucose/dl pre-breakfast decreased with insulin to 149 mg glucose/dl post breakfast one hour later and on another day a blood glucose of 404 mg glucose/dl pre-breakfast decreased with insulin to 130 mg glucose/dl post-breakfast two hours later. These rapid and large decreases in blood glucose are sufficient to cause the adrenergic signs and symptoms of hypoglycemia he claimed to experience and that can occur in a diabetic patient whose body has grown accustomed to very high average blood glucose levels.

The patient's blood glucose values that were downloaded from his blood glucose meter were higher than predicted from the National Glycohemoglobin Standardization Program formula which is based on hemoglobin A1c values measured by HPLC. Almost all of his hemoglobin A1c values were measured on the DCA 2000 Seimens/Bayer Analyzer in an outpatient setting on finger-stick samples of blood and not by HPLC. For example, at ages 17 3/12 and 17 8/12 his hemoglobin A1C values were 10.1% and 10.4%, respectively, at visits to the diabetes clinic. According to the National Glycohemoglobin Standardization Program formula these hemoglobin A1c values should reflect average blood glucose values of 243 and 252 mg glucose/dl, respectively.

However, the average of blood glucose values measured during the 14 days prior to these two clinic visits immediately before and after breakfast, before lunch, before supper and before bedtime with his blood glucose meter were 337 ± 116 glucose/dl (N = 24 values) and 315 ± 118 glucose/dl (N = 28 values) (means ± SD), respectively, which are much higher than average blood glucose values predicted by the National Glycohemoglobin Standardization Program formula. There are several reports that the DCA 2000 Analyzer slightly underestimates hemoglobin A1c values compared to hemoglobin A1c values measured by HPLC. In any case, the patient's hemoglobin A1c values and blood glucose values indicate the patient's glycemic control was poor.

SUPPLEMENTARY DATA

At 18-19 years of age the patient's glycemic control had improved somewhat. His liver size decreased and he had started to progress through puberty. His liver was still enlarged. Its lower edge was still 2 cm above his umbilicus. Blood transaminase values were normal.

DNA sequencing results of the exons of genes that encode enzymes of glycogen metabolism in the Mauriac Syndrome patient:

G6PC gene – Glucose-6-phosphatase (G6Pα): Except for an A/G variation in exon 3 at nt 372 (NM_021176) that resulted in a synonymous codon change (A124A) in the G6Pα protein the exonic sequence of G6PC is normal.

G6PC3 gene – Glucose-6-phosphatase β (NM_138387): There is a known G to C variation (rs1046770) in the 3' untranslated region at nt 1428 of mRNA (NM_138387). All other exons were normal.

SLC37A4 gene - Glucose-6-phosphate transporter (G6PT): The sequences of all exons of this gene were that of the wild type.

AGL gene – Glycogen debranching enzyme: The patient possesses three known variations in the AGL gene. One is an A to G variation noncoding SNP (rs2307130) that is 10 nucleotides upstream of the ATG start codon of AGL in exon 3. A second mutation is an A to G homozygous mutation in intron 8 (SNP rs634880) 18 nucleotides upstream of exon 9. The third variation (rs17121464) is in exon 10 and converts an arginine to a glutamine (R387Q). Exon 10 was sequenced in 33 type 1 diabetics with normal size livers. Two individuals possessed the minor allele. One was a Caucasian and the other one was a patient whose father is Japanese and mother is Caucasian. The minor allele of SNP rs17121464 has a frequency of 0.042 in Caucasians (N = 48 chromosomes) (NCBI website). The frequency is 0.333 in Asians (2).

PYGL gene – Liver glycogen phosphorylase: The patient possesses three known variations in the PYGL gene. There is a known variation at the splice site of intron 15 and exon 16. This is a heterozygous deletion of an A nucleotide in the splice acceptor site of exon 16 of the gene (SNP rs33993827/ CCCCAAAGG/ CCCCAAGG). This variation was present in 16 of 32 type 1 diabetes (50%) patients who do not have an enlarged liver or Mauriac Syndrome. Patients' DNA samples selected for DNA sequencing were randomly selected from ~ 1000 patient samples. In exon 9, there is a heterozygous C to T variation (SNP rs2075643). This variation is a synonymous mutation and does not cause an amino acid change in the glycogen phosphorylase protein. There is a known heterozygous G to C variation at position 172 in exon 20 (SNP rs1042266) seven nucleotides downstream of the stop codon in the 3' UTR and a known heterozygous T deletion (SNP rs3216001) at nt176 TGAACTTGAACATTTTT 3 nucleotides downstream of the G to C variation (rs1042266). We sequenced PYGL exon 20 in DNA samples of 31 patients with type 1 diabetes and found 12 individuals that have the T deletion at rs3216001 and 19 samples had the wildtype sequence. The SNP rs1042266 was present in five of 31 of the patients with type 1 diabetes. The five patients that have the rs1042266 variation were also found to have rs3216001. Since the variations are common in the PYGL gene and do not cause an amino acid change in the protein, it is highly unlikely they are pathogenic.

SUPPLEMENTARY DATA

PHKA2 gene – Liver glycogen phosphorylase kinase α subunit: The sequences of all 36 exons were normal. We found three previously unreported intron variations. One is a heterozygous deletion of a T in a string of 22 Ts in intron 3 142-164 nt downstream of exon 3. A second intron mutation is a C to T homozygous mutation in intron 12 located 38 nt downstream of exon 12. A third variation is a homozygous C to T variation in intron 31, 20 nucleotides upstream of exon 32.

REFERENCES

1. Kerouz N, El-Hayek R, Langhough R, MacDonald MJ. Insulin doses in children using conventional therapy for insulin dependent diabetes. Diabetes Res Clin Practice 1995;29:113-20.
2. Veiga-da-Cunha M, Gerin I, Chen Y-T, et al. A gene on chromosome 11q23 coding for a putative glucose-6-phosphate translocase is mutated in glycogen-storage disease types Ib and Ic. Am J Hum Genet 1998;63:976-83.