Insulin Mutation and Neonatal Diabetes

Insulin is a hormone that functions in the regulation of blood glucose level. In some cases of neonatal diabetes, the gene coding for the insulin protein has a nucleotide-pair substitution mutation that alters the protein structure enough to cause it to malfunction. By sequencing an individual's whole genome, doctors can use that DNA sequence information to diagnose diseases and identify new treatments. For example, the insulin gene sequence of a patient with neonatal diabetes can be analyzed to determine if it has a mutation and, if so, its effect.

Imagine you are a medical geneticist presented with three infant patients, all of whom have a nucleotide-pair substitution in their insulin gene. You are trying to determine the effect of the mutation on the amino acid sequence of the insulin protein. To identify the mutation in each patient, you can compare his or her individual insulin DNA sequence to that of the wild-type DNA.

1. [SP 1] Describe how you could identify which amino acids are altered in the patient's insulin protein. (Identifying the codons that have been changed will tell you which, if any, amino acids are altered.)

The sequences of the wild-type DNA and the patients' DNA, arranged in codons, are shown in Figure 1. The sequences show the codons for amino acids 35–54 (of the 110 amino acids in insulin), so the start codon (AUG) is not present.

Wild-type cDNA	5′-CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TTC TTC TAC ACA CCC AAG ACC-3′
Patient 1 cDNA	5′-CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TGC TTC TAC ACA CCC AAG ACC-3′
Patient 2 cDNA	5′-CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TCC TTC TAC ACA CCC AAG ACC-3′
Patient 3 cDNA	5′-CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TTC TTG TAC ACA CCC AAG ACC-3′

Figure 1 Sequences for wild-type DNA and DNA from three patients.
Data from N. Nishi and K. Nanjo, Insulin gene mutations and diabetes, *Journal of Diabetes Investigation* 2:92–100 (2011).

2. [SP 1, SP 4] Comparing each patient's cDNA sequence to the wild-type cDNA sequence, circle the codons where a nucleotide-pair substitution mutation has occurred.
   a) Identify any differences between the amino acid that will be made by the codon with the mutation in each patient's insulin sequence and the amino acid made by the codon in the corresponding wild-type sequence. Note that the sequence provided are from the *coding* (nontemplate) strand, so to convert it to mRNA you just need to change T to U. (Patient 1: mutation (TGC) codes for cysteine (Cys); wild-type sequence (TTC) codes for phenylalanine (Phe) in every case. Patient 2: mutation (TCC) codes for serine (Ser). Patient 3: mutation (TTG) codes for leucine (Leu).)
   b) Identify each patient's nucleotide-pair substitution mutation as silent, missense, or nonsense. Justify your response. (Patient 1: missense mutation because the wrong amino acid will be incorporated into this position in the insulin protein. Patient 2: missense mutation because the wrong amino acid will be incorporated into this position in the insulin protein.

Patient 3: This patient has a missense mutation because the wrong amino acid will be incorporated into this position in the insulin protein.)

3. [SP 1, SP 6] Compare the structure of the amino acid you identified in each patient's insulin sequence to that of the corresponding amino acid in the wild-type insulin sequence. Provide an explanation for how the change of amino acid in each case might have affected the insulin protein produced. (For all three patients, the wild-type codon that corresponds to the mutation codes for the amino acid phenylalanine (Phe). Phenylalanine has a nonpolar side chain and is hydrophobic. Patient 1: The mutant codon codes for cysteine (Cys), which has a weakly polar side chain, so it could change the shape of the insulin protein. Patient 2: The mutant codon codes for serine (Ser), which is polar and hydrophilic, so it is likely to change the shape of the insulin protein. Patient 3: The mutant codon codes for leucine (Leu), which is nonpolar and hydrophobic, so it might not have much effect on the shape of the insulin protein.)