Patient Name: Jonathan Sandman  
Case Investigation #: 587291

Investigators,

We now realize that we need more information about the patient's sleep pattern. For this we requested that the patient keep a personal sleep-wake log that you will have access to during this portion of your investigation.

We think it might be time for you to focus on the protein cycle that helps regulate circadian rhythms. Recent research has shown that one of the more important proteins in this cycle is called Period2 (PER2). This PER2 protein can be modified in research animals, and we have included a recent study by a group that explored this mutation in mice. See if you can interpret the results of the study, and if they have any importance in diagnosing our patient. Below is the breakdown of the materials we were able to collect for you:

- Patient records
  - Self-recorded sleep log

- Additional information
  - Hospital Info Sheet: Western Blots
  - PER2 Mutant Data Analysis and Actograms

After looking through these files complete the checkpoint and we will supply you with the next set of case materials.
A Western Blot is used to detect certain proteins and their relative amounts within a tissue sample. This process, sometimes referred to as protein immunoblot, also helps scientists to discover the size of a specific protein.

In the first step of this technique, gel electrophoresis is used to separate the proteins based on their molecular weight (Figure 1). There are wells in the gel and each of these is loaded with a protein sample using a pipette. The first and last wells are typically loaded with a prepared mixture of proteins that have defined molecular weights. This “ladder” allows scientists to compare the sample protein sizes with those that are already known. When the power supply is turned on and the voltage is passed through the gel, the samples begin moving out of the wells towards the positive end of the gel. Protein samples that are smaller migrate more quickly in their lanes than the larger ones do. After a certain amount of time, the power supply is turned off and the samples are ready for the next step.

Next, the proteins are transferred to a membrane, which is a special type of blotting paper. Typically the membrane is made of nitrocellulose, but other types can be used depending on the experiment. After the proteins have been transferred they are still not visible. At this stage a special dye can be added which makes the protein visible and helps the experimenter verify that the transfer to the nitrocellulose membrane was uniform.

To get clearer results and eliminate false positives, the blot is then placed in a solution of generic proteins and detergent. These generic proteins bind to all of the binding sites on the membrane that are not already bound by the original samples of protein, a process called blocking. This prevents unwanted binding of antibodies during the next steps of the process.
In order for specific proteins to be detected or “probed” on the Western, the experimenter must conduct a 2-step process (Figure 2). First, a primary antibody is added that binds to the protein of interest. Then, a secondary antibody with an attached fluorescent enzyme (probe) binds to the primary antibody and allows the experimenter to detect the protein. After the antibodies have had time to bind, it is critical that any unbound antibodies be washed away so that only the probed protein remains. The membrane is then incubated with a substrate that reacts with the probe. This produces a color reaction resulting in visibility of the protein of interest (Figure 3). Those bands that have an intense color reaction have a higher protein concentration than bands with a lower color reaction. This is because there is more antibody/enzyme probe attached in these areas allowing more color to be present. Finally the probed protein bands are photographed for analysis.

Western Blot diagram: http://www.bio.davidson.edu/courses/genomics/method/westernblot.gif
Antibody diagram: http://tinyurl.com/66muebl
Some information obtained from:
http://www.bio.davidson.edu/courses/genomics/method/Westernblot.html
http://www.piercenet.com/Proteomics/browse.cfm?fldID=8259A7B6-7DA6-41CF-9D55-AA6C14F31193
PER2 Mutant S662G Data Analysis

Xu et al. Cell. 2007

S662G is a modified PER2 protein found in some strains of mice. In order to study S662G’s effects on the circadian cycle of these mice, researchers collected the data seen here:

A Western Blot for the S662G protein taken from the liver cells of the mice was run every few hours and is shown in the lower right. The Tubulin protein was used as a control marker in each well.

The comparison of the PER2 protein level to the Tubulin control is graphed in the bottom left (for example, a value of .4 at time 0 means the PER2 blot was 40% the intensity of the Tubulin control). The bars on each point represent the margin of error for the analysis across multiple Westerns.

Finally, a group of mice with the modified S662G protein had their circadian cycle monitored over several days by tracking their activity on a cage wheel. The mice were first given a normal light-dark cycle for one week (the top portion in the chart on the next page) and were then switched to an all dark cycle for the next two weeks in order to document any changes to their circadian rhythm in the absence of external stimuli.
Recorded actogram for mouse with S662G Mutation

Xu et al. Cell. 2007
S662WT (Wild-Type) is the natural PER2 protein found in some strains of mice. In order to study S662WT’s effects on the circadian cycle of these mice, researchers collected the data seen here.

A Western Blot for the S662WT protein taken from the liver cells of the mice was run every few hours and is shown in the lower right. The Tubulin protein was used as a control marker in each well.

The comparison of the PER2 protein level to the Tubulin control is graphed in the bottom left (for example, a value of .6 at time 0 means the PER2 blot was 60% the intensity of the Tubulin control). The bars on each point represent the margin of error for the analysis across multiple Westerns.

Finally, a group of mice with the S662WT protein had their circadian cycle monitored over several days by tracking their activity on a cage wheel. The mice were first given a normal light-dark cycle for one week (the top portion in the chart on the next page) and were then switched to an all dark cycle for the next two weeks in order to document any changes to their circadian rhythm in the absence of external stimuli.

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**Graph:**

- X-axis: Time (0, 6, 12, 16, 20, 24 hours)
- Y-axis: Protein levels
- Points indicate PER2 protein levels over time for WT mouse.
- Bars represent the margin of error for each measurement.
Recorded actogram for mouse with S662 Wild Type (WT) condition

Xu et al. Cell. 2007
S662D is a modified PER2 protein found in some strains of mice. In order to study S662D’s effects on the circadian cycle of these mice, researchers collected the data seen here.

A Western Blot for the S662D protein taken from the liver cells of the mice was run every few hours and is shown in the lower right. The Tubulin protein was used as a control marker in each well.

The comparison of the PER2 protein level to the Tubulin control is graphed in the bottom left (for example, a value of .9 at time 0 means the PER2 blot was 90% the intensity of the Tubulin control). The bars on each point represent the margin of error for the analysis across multiple Westerns.

Finally, a group of mice with the modified S662D protein had their circadian cycle monitored over several days by tracking their activity on a cage wheel. The mice were first given a normal light-dark cycle for one week (the top portion in the chart on the next page) and were then switched to an all dark cycle for the next two weeks in order to document any changes to their circadian rhythm in the absence of external stimuli.
Recorded actogram for mouse with S662D Mutation
Xu et al. Cell. 2007
Sleep Log

Reported: Friend of Patient  Log type: Self Report

Activity

School Vacation

June

2012