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Review Article

Phenylketonuria: Disease Characteristics, Symptoms and Cause of Disease

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Abstract

Phenylketonuria is a genetic defect which causes the accumulation of phenylalanine in the body. This metabolic disorder can be detected from birth and is rare. Phenylalanine is one of twenty essential amino acids which the human body needs in order to function properly and grow (Scriver, 2007). This amino acid makes up proteins in the body, which without; the body will not function normally. However, the accumulation of this amino acid can be detrimental to the health.

Keywords: Phenylketonuria; Phenylalanine; Health

Introduction

A defect in the gene which produces an enzyme called phenylalanine hydroxylase causes Phenylketonuria. This enzyme breaks down the amino acid, and without the enzyme the amino acid builds up in the body causing problems to the health. The symptoms of this genetic disease may be mild or severe. Symptoms include, epilepsy, hyperactivity, behavioural issues, tremors, stunted growth, and skin conditions like eczema, musty breath odour, skin odour or even urine odour. More severe symptoms occur when this disease is detected at a later stage. Symptoms such as irreversible brain damage and seizures may be seen at later stages [1]. It can only be detected in infants a few months after birth. This is done through a blood test. Blood is taken from the baby's heel and tested for the genetic disorder [2]. A hypothetical animal model and gene therapy will be designed for Phenylketonuria.

The PAH gene is responsible for sending messages to produce the enzyme phenylalanine hydroxylase. PAH mutations either delete the DNA from the gene in small amounts or disrupt the gene from making the enzyme which breaks down the amino acid phenylalanine. Figure 1 shows the location of this gene on chromosome 12q. This is an autosomal recessive inheritance meaning that two

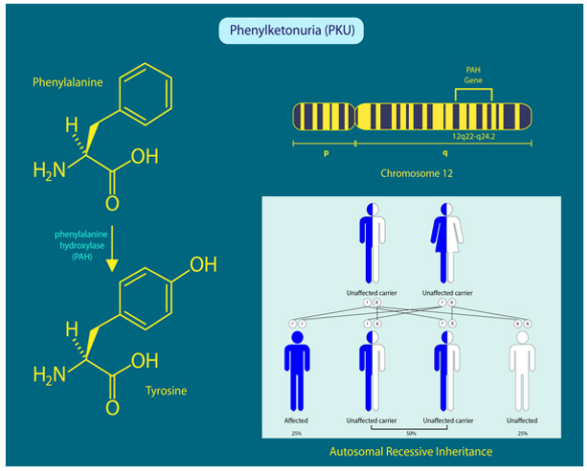

Figure 1: PAH gene on chromosome 12q (Scriver, 2007).

copies of this mutated gene have to be present in order to develop the disease [3].

Hypothetical disease model

The category of animal model produced

The use of mouse to study genetics in humans has been used for a long time due to the fact that these two species have a very similar genetic and physiological makeup. Mouse have characteristics such as short life cycle, short gestation period and the ability to produce an abundance of offspring, hence making them almost perfect as models for human disease and preclinical research [4]. Mice also contain 98% of genes as humans. This will be an experimental model because it will be induced artificially in the laboratory using a mouse model. In order to achieve the mouse model, various steps need to take place to manipulate the genome of this animal model [5].

The genetic modification strategy and approach

A disease driven, directed genetics strategy must be used for this experiment because, Phenylketonuria is a genetic disease which will need to be mimicked in the mouse model. This genetic disease involves the disruption of a gene which produces an enzyme which can break down the amino acid phenylalanine [6]. Without this enzyme, phenylalanine accumulates in the body and becomes detrimental over a long period. The genes of the mouse model must be targeted and disrupted in order to stop producing the enzyme, phenylalanine hydroxylase [7].

Transgene identity

In the case of Phenylketonuria, a knock-out mouse can be used to test possible cures for this metabolic defect. An engineered Phenylketonuria gene needs to be introduced into the mouse at a specific gene locus through homologous recombination (Chromosome 12q). Using gene targeting, this genetic disorder can be mimicked into the mouse model. The mutated version of the exon needs to replace the normal copy through homologous recombination. In order to create this replacement, the recombination of the two flanking arms needs to take place [4]. The mutated gene needs to be designed into a homology arm. Once the sequence in the target gene is exchanged with the vector sequence, the mutation will be introduced into chromosome12q. Due to the fact that this is a gene targeting experiment, the replacement vector will disrupt the essential coding exon. Selection of recombinant clone can happen by inserting a positive drug resistant marker. The Homologous recombination in embryonic stem cells will result in a gene being inactive, in this case the gene which produces the enzyme to break down the amino acid will need to be inactive [8].

Transformation methodology - embryonic stem cell

Embryonic stem cells are harvested from the animal donor (mouse) followed by introducing (transfection) the transgene into the embryonic stem cells. After incorporating the gene with the desired mutation for the human disease Phenylketonuria, a pre-implantation embryo must be created by inserting the embryonic stem cell into a blastocyst of a newly isolated blastocyst. A surrogate mouse must be selected [4]. Administer follicle stimulating hormone in order for the surrogate mouse to ovulate. Once ovulated, the selected embryo must be cultured and transferred into the reproductive tract of this surrogate mouse. The surrogate mouse must copulate with a fertile male in order to achieve fertilisation. The resulting progeny (offspring) must be screened to identify and select the desired genes. The founder chimeric progeny will have both wild-type coat colour (black) and treated coat colour (brown), these mice will be carrying cells from both mouse strains. Screening and breeding of these mice needs to take place in order to create homozygous mice [9].

Hypothetical gene therapy

Prerequisites for gene therapy

Phenylketonuria results from a gene mutation in the alleles of the gene which produces phenylalanine hydroxylase on chromosome 12q. The gene which is mutated is known as well as its location on the chromosome. This disease is an in-born defect, therefore it affects babies from 6 weeks old and can be detrimental to the health if not taken care of from an early stage [10]. Studies have showed that this disease affects infants and can cause serious health issues during adulthood. The build up of the amino acid phenylalanine in the body is toxic and affects the blood, liver and mainly the tissues of the brain, causing mental retardation. The mutation of the PAH gene stops the production of the enzyme. The failure to hydrolyze the phenylalanine with the enzyme phenylalanine hydroxylase to form tyrosine can lead the accumulation of the amino acid in the body. When the body cannot produce tyrosine, this will affect the production of proteins as this is a building block of protein. This is an autosomal recessive inheritance, meaning that both parents need to have this mutated gene in order to pass

it on to their offspring [11]. If only one parent carries this mutated gene, it will not be passed on to the offspring. Transgene in affected cells will not kill the affected cells; it will rather regulate the accumulation of amino acid [12].

Administration of gene therapy

Somatic gene therapy will only cure the patient suffering from Phenylketonuria and not the descendants of the patient. This is due to the fact that it is an autosomal recessive disease. Germline gene therapy will have to be considered if descendants are taken into considerations. However for this study, the main focus is the single patient. The affected issues cannot be removed from the body therefore the gene therapy will have in be *in-vivo*. A direct injection will most likely be suitable for this type of disease [13].

Strategy and transfer methodology

Since the gene which produces the enzyme phenylalanine hydroxylase has a defect, the best option will be to repair the gene. CRISPR (similar to knock-in) is a method used to repair genes; it is a genome-editing technique which allows scientists to select a specific gene and add specific components to repair the gene. In order to allow the vector to enter the affected cells a non-viral transformation method will be used known as naked DNA injection. Non-viral methods are cost effective and safer due to the fact that using a virus could implicate matters by affecting the host. Naked DNA is free DNA, which is not surrounded by either lipids or proteins. Naked DNA consists of components of sequences required for the host genome [15]. When performing gene therapy three main components need to be taken into consideration, these are the vector, the gene of interest and the target site. This is a form of intramuscular injection. This method of injection can integrate about ~15 kb of transgene. There are several methods for administrating non-viral vectors into the affected tissue:

- **Needle:** The genetic material is administered using a needle into the tissue; however this can be risky due to the nucleases in the serum that can degrade the genetic material of interest. (Direct delivery of Naked DNA) [14].
- **Ballistic DNA:** Using a Gene gun, the genetic material of interest is inserted precisely into its desired location.
- **Electroporation:** This is the application of an electric field in order to open the pores for the gene of interest to enter exactly where needed [13].
- Different methods can be used for administration if the needle injection fails.

Transgene identity

The transgene is associated with stable expression because it is from an inherited disease condition. The plasmid DNA will be integrated into the chromosome 12. This DNA will get passed on to the next generations of cells [14]. When it comes to stable expression it is better to use linear DNA, however the uptake is slower than that of supercoiled DNA. An episomal (genome-integrated DNA vector containing an origin of expression must be used. A one-on-one method must be used, where the specific DNA sequence will be targeting the gene which is mutated and does not produce the enzyme [15].

Conclusion

The use of animal models in preclinical studies is very important, because they have a lot of genetic similarities to humans. Diseases can be studied and therapies can be tested on animals before clinical trials on humans. The main reason for preclinical studies on animal models is because of their short life cycle and their ability to produce a large number of offspring. Animals are used to get diagnosis, treatment and prevention of human diseases. Gene therapy is very technical, yet helpful. If it wasn't for animal models a lot of people would lose their lives for research purposes. Medications are tested on humans only after successful results are obtained from animal models which mimic the human disease through genetic transformations. It can prolong the lives of those who are born with genetic defects and also stop the gene from being passed on to the next generations.

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