Homocystinuria is an autosomal recessive disorder affecting approximately 1 in 350,000 people worldwide. It is characterized by skeletal, nervous system, and vascular abnormalities, such as delayed developmental milestones, myopia, dislocation of the eye lens, osteoporosis, mental retardation, and increased risk of blood clotting. Major causes of homocystinuria are mutations in the enzyme cystathionine β-synthase (CBS), which catalyzes the condensation of serine and homocysteine to cystathionine, an intermediate in cysteine synthesis (Figure 1).

**Figure 1.** Reaction pathway for the synthesis of the amino acid cysteine. Synthesis of cysteine begins with CBS-catalyzed condensation of homocysteine and serine. 

Over 150 mutations have been identified affecting CBS. These mutations cluster around three areas of the enzyme: the heme binding site, the PLP and substrate binding active site, and the dimer interface. At locations other than the PLP binding site, vitamin B6 treatment is ineffective and homocystinuria cannot be managed. Thus, research is being conducted to find other treatments for CBS-related vascular abnormalities due to mutations at other areas of the CBS enzyme.

**MUTATIONS IN CBS ASSOCIATED WITH HOMOCYSTINURIA, CLUSTER IN THREE REGIONS OF CBS, RESULTING IN DIFFERENTIAL EFFICACY OF VITAMIN B6 TREATMENT**

![Diagram of CBS enzyme with mutations indicated](image)

The CBS gene, found on chromosomes 21, provides instructions for the enzyme cystathionine β-synthase. This enzyme catalyzes the condensation of serine and homocysteine to form cystathionine. CBS is a pyridoxal 5'-phosphate (PLP) and heme-dependent enzyme regulated by S-adenosylmethionine (SAM). The N-terminal domain is the catalytic region, containing both the PLP and heme cofactors and catalyzing the synthesis of cystathionine. The iron-binding site and heme binding PLP cofactor at active site and heme binding domain structure are shown in Figure 2.

**Figure 2.** Each subunit of CBS contains two domains. CBS is a pyridoxal 5'-phosphate (PLP) and heme cofactor and cystathionine synthase (CBS) enzyme. The N-terminal domain is the catalytic region containing both the PLP and heme cofactors, while the C-terminal domain is the regulatory region binding SAM and Cbs-b.

Homocystinuria can lead to several cardiovascular defects such as increased carotid plaque thickness, known as atherosclerotic plaques, and intravascular thrombosis, the formation of a blood clot that obstructs blood flow through the circulatory system. A primary research goal is to understand how mutations in CBS, an enzyme found in muscle tissue, may lead to vascular abnormalities by identifying changes in embryonic vascular development in zebrafish (Danio rerio) lacking expression of the Cbs-b isoform. Zebrafish are used as a model to study the effects of CBS mutations due to their small size, closed circulatory system, and rapid development. In addition to changes in physical and chemical changes in human vascular development, homocystinuria is associated with vascular defects in zebrafish, as shown in Figure 3, which illustrates mutations associated with homocystinuria.

**Figure 3.** Coordination of PLP and heme cofactors in the N-terminal domain of CBS. The PLP cofactor (orange) is located within the active site of the enzyme and is converted into CBS by cysteine-119 (blue). The heme cofactor (red) is reversibly bound to CBS by coordination with heme-iron (blue) and histidine-65 (cyan). The iron (peach) rests in the center of the heme ring. Figure generated using Jmol and PDB file 1JBQ from Meier et al.

To investigate the role of CBS enzyme in vascular development, morpholino technology was used to reduce the expression of the Cbs-b isoform in zebrafish, as shown in Figure 4. Morpholino oligos (MO) are synthetic oligonucleotides that use DNA bases on a phosphorodiamidate backbone. Once injected into the fertilized egg, an MO specifically binds to its target mRNA, blocking mRNA splicing or translation of the mRNA into its respective protein product. The MO’s were synthesized to be complementary to different regions of the cbs-b mRNA. Injection of MO’s into fertilized eggs prevented the translation of the mRNA into its respective protein product. The MO’s were synthesized to be complementary to different regions of the cbs-b mRNA. Injection of MO’s into fertilized eggs prevented the translation of the mRNA into its respective protein product. The MO’s were synthesized to be complementary to different regions of the cbs-b mRNA. Injection of MO’s into fertilized eggs prevented the translation of the mRNA into its respective protein product. The MO’s were synthesized to be complementary to different regions of the cbs-b mRNA. Injection of MO’s into fertilized eggs prevented the translation of the mRNA into its respective protein product. The MO’s were synthesized to be complementary to different regions of the cbs-b mRNA. Injection of MO’s into fertilized eggs prevented the translation of the mRNA into its respective protein product. The MO’s were synthesized to be complementary to different regions of the cbs-b mRNA. Injection of MO’s into fertilized eggs prevented the translation of the RNA into the respective mRNA product. Injection of MO’s into fertilized eggs prevents the translation of the mRNA into its respective protein product. Injection of MO’s into fertilized eggs prevents the translation of the RNA into the respective mRNA product.

**Figure 4.** Quantification of two different injection experiments for the ISV phenotype observed in Figures 6 and 7.

In zebrafish, following the initial formation of the dorsal artery and posterior cardinal vein, new blood vessels extend from these preexisting vessels. These budding vessels, the segmental vessels (ISVs), are among the first angiogenic vessels to form. Since zebrafish are a well-established model for vascular development, scientists may be able to observe the vascular development of the ISVs in zebrafish lacking functional CBS enzyme. Blood vessel formation can be monitored in developing embryos by staining for the presence of the VEgf receptor 2 (VEGFR2) mRNA. VEGFR2 is expressed on the surface of endothelial cells to direct vessel formation in response to the growth factor. VEGF, sequestered by neighboring somites.

**CONCLUSION**

In this study, morpholino technology was used to prevent the expression of the cbs-b gene in zebrafish and vascular development was assessed in embryos to determine if any phenotype might arise as a result of the loss of CBS. A morpholino oligonucleotide complementary to the mRNA of cbs-b was injected into fertilized eggs of the zebrafish, inhibiting proper splicing and/or translation of the protein product of the CBS gene. As shown in Figure 9, Zebrafish embryos lacking the CBS enzyme exhibited a death rate with ISVs in the trunk of the developing embryo. No apparent effect on the number of ISV segments or vessel sprouting was apparent. This disruption in the development of the ISVs suggests that mutations in the CBS gene has an affect on vascular growth and function. The continuation of this work will hopefully lead to more information regarding vascular abnormalities caused by mutations in CBS. As research continues, scientists hope to create a model system to study the effects of CBS in vascular development, in order to better understand CBS deficiencies in humans. Eventually, researchers hope to develop multiple treatments for various CBS related diseases such as homocystinuria.