cryobio

Additional Agreement to Use Donor CB 493

We, _____ (Recipient), and _____ (Partner, if applicable), specifically request and accept frozen semen from Cryobio donor CB 493. We understand that this Additional Agreement is an additional part of the Sperm Use Agreement specific to donor CB 493. We have received genetic test results on this sperm donor, and we understand that donor CB 493 has been found to be a carrier of the following recessive genetic conditions:

Gitelman Syndrome,

Nephrotic Syndrome (NPHS2-Related)/Steroid-Resistant Nephrotic Syndrome,

Neuronal Ceroid-Lipofuscinosis (CLN3-Related),

and

Ornithine Aminotransferase Deficiency.

Why carrier status is important: Carriers of genetic diseases have changes, called pathogenic variants or mutations, in a specific gene or multiple genes. Most of the genetic diseases that the Cryobio donors are tested for are inherited in an autosomal recessive pattern. Typically, we all have two copies of every gene---one from the egg source and one from the sperm source. Autosomal recessive diseases require a mutation in both copies of the same gene in order for it to cause disease. Therefore, individuals who carry just one mutation in a gene that causes recessive disease are 'carriers' of that specific disease. Carriers of most of the genetic diseases Cryobio donors are tested for do not show symptoms of the disease, i.e., they are asymptomatic. Some diseases tend to occur more in certain ethnicities, and some tend to occur evenly in all ethnicities. Most individuals are carriers for at least one if not multiple recessive genetic diseases.

Carrier status is important because if both the egg source and the sperm source are carriers for pathogenic variants or mutations in the same gene, then there is a 1 in 4 chance of the resulting child having that specific disease; a 2 in 4 chance of the resulting child being a carrier for that specific disease; and a 1 in 4 chance of the resulting child being neither a carrier nor having that specific disease. Some of the diseases Cryobio donors are tested for have genotype-phenotype correlation, meaning that specific genetic pathogenic variations (the genotype) in a specific gene can be predictive of the type of specific disease that may present in the individual (the phenotype), but not all do.

Gitelman syndrome (SLC12A3 gene):
Gitelman syndrome is an autosomal recessive, pan-ethnic disease caused by pathogenic variants in the gene SLC12A3. In this disease, the kidney does not retain necessary particles/ions, causing an imbalance in the body. Symptoms usually begin in late childhood or adolescence, and include muscle spasms or cramps, tingling sensations, joint pain, and fatigue. Most patients have mild symptoms, but severe ion imbalances could lead to seizures or heart arrhythmias. With treatment, including dietary management, patients have a normal life expectancy. It is not currently possible to predict the severity of symptoms based on the variants inherited. Studies have suggested carriers of Gitelman syndrome may have a lower blood pressure compared to that of the general population. However, carriers are not expected to have clinical problems or symptoms of the disease.

Of Note: There has been one case report of the possibility of digenic inheritance in an individual with Gitelman syndrome. Digenic inheritance means genetic variants in two *different* genes combined cause a

disease. In this case report, it is *suspected* that one SLC12A3 and one CLCKNB variant were the cause of disease. However, the pathogenicity of the CLCKNB gene could not be confirmed. More evidence is needed to conclude digenic inheritance as the cause of Gitelman syndrome. As an extra precaution, recipients should consider testing the recipient (or egg source if different) for CLCKNB carrier status in addition to SLC12A3 carrier status.

Nephrotic Syndrome (NPHS2-Related/Steroid-Resistant Nephrotic Syndrome (NPHS2 gene): Nephrotic syndrome (NPHS2-related), also known as steroid-resistant nephrotic syndrome, is a genetic disease caused by pathogenic variations or mutations in the NPHS2 gene. It is inherited in an autosomal recessive inheritance pattern. Individuals from all ethnicities can be carriers for nephrotic syndrome (NPHS2-related)/steroid-resistance nephrotic syndrome. Onset is usually during childhood or adolescence. Symptoms include loss of protein in the urine, which results in progressive kidney failure. Death will occur without a kidney transplant, usually by adolescence; however, many patients are cured after kidney transplant. Several specific variants may be associated with an earlier or later age of onset of disease, but not all variants are known to have a genotype-phenotype correlation.

Of Note: The specific variant identified in donor CB 493 (p.R229Q) is a common pathogenic variant which is known to cause disease only when in combination with specific variants in the NPHS2 gene, but it does not cause disease with *all* other variants of the NPHS2 gene. Regardless, if the egg source were also identified to be a carrier of a NPHS2 variant, we strongly encourage and recommend review of the information with a genetics professional to better understand the risks of disease to children. Carriers are not expected to show symptoms of this disease.

Neuronal Ceroid-Lipofuscinosis (CLN3-Related) (CLN3 gene): Neuronal ceroid-lipofuscinosis (NCL) is a term used to refer to a group of 13 genetic lysosomal storage disorders that primarily affect the nervous system. The 13 different subtypes are classified primarily based on their underlying genetic cause and vary in symptoms and age of onset. The genetic changes that cause NCLs disrupt the cells' ability to dispose of certain wastes. In NCLs, buildup of these specific wastes occurs most dramatically in nerve cells (called neurons) of the brain, but also in other tissues of the body, such as the eyes. This buildup of these wastes causes deterioration of the brain, neurological impairment, seizures, eye disease, and other symptoms that may vary by subtype. Neuronal Ceroid-Lipofuscinosis (CLN3-related) is the form donor CB 493 is a carrier for, and it is caused by pathogenic variants in the gene CLN3. NCL (CLN3-related) is the most common form of NCL and is also known as/referred to as Batten disease.

NCL (CLN3-related) is inherited in an autosomal recessive pattern, and it has been reported in patients from different ethnicities around the world. Most CLN3-caused neuronal ceroid-lipofuscinosis results in a juvenile form, in which symptoms begin between 4 and 10 years of age. Clinical features include progressive visual loss which proceeds to blindness in childhood. Neurologic and psychiatric symptoms include seizures, difficulty speaking, intellectual disability, psychosis or dementia, and ataxia (loss of full control of body movements) leading to an inability to walk. Affected individuals often die between the ages of 20 and 40. Some patients have a milder form with less neurologic involvement.

Currently, there are medications to help treat symptoms of NCL (CLN-3 related) (for example medications are available to help reduce/treat seizures, physical therapy to assist with ataxia, etc.), but there is no available treatment to cure the underlying disease itself. Severity of disease can vary widely, and it is not currently possible to predict the severity of disease for each individual with NCL (CLN-3 related).

Ornithine Aminotransferase Deficiency (OAT gene): Ornithine aminotransferase (OAT) deficiency (also known as gyrate atrophy) is an autosomal recessive disorder caused by pathogenic variants in the gene OAT. While affected individuals have been reported worldwide, the disease has an increased prevalence in Sephardic Jewish individuals from Iraq and Syria and individuals of Finnish descent due to the presence of founder mutations.

The OAT gene codes for a protein that aids in protein metabolism. When the OAT gene is not functioning properly, it leads to a buildup of a molecule in the body called ornithine. Buildup of ornithine is thought to result in the symptoms/features of OAT deficiency. Clinical features include the onset of night blindness and myopia (near-sightedness) in the first decade of life, with progression to blindness in adulthood. Cataract development usually occurs in adolescence or early adulthood. Most individuals with OAT deficiency have no other symptoms than vision loss, others may present with muscle abnormalities/muscle weakness or other symptoms. Life expectancy and intelligence are typically normal. Vitamin supplementation and dietary restriction to limit ornithine build up may aid in slowing the progression of vision loss. There is currently no way to predict severity of disease based on the genetic variants present in affected individuals.

Carrier status frequency:
Carrier status frequency is the chance of an individual being a carrier for a genetic condition based on their ethnicity alone prior to any genetic screening. If an individual tests negative as a carrier for a condition or conditions, then the chance of being a carrier is significantly decreased. This remaining risk is known as residual risk, meaning "what is the risk of being a carrier even after negative genetic testing?" Residual risk data is available directly through sema4's website, sema4.com, or can be requested from Cryobio.

Gitelman syndrome (SLC12A3 gene) carrier status frequency in different ethnicities, from Sema4's website:

Worldwide	1 in 82
African	1 in 138
Ashkenazi Jewish	1 in 121
East Asian	1 in 28
Finnish	1 in 239
European (Non-Finnish)	1 in 73
Native American	1 in 131
South Asian	1 in 145

Nephrotic syndrome (NPHS2-Related)/steroid-resistant nephrotic syndrome (NPHS2 gene) carrier status frequency in different ethnicities, from Sema4's website:

Worldwide	1 in 16
African	1 in 77
Ashkenazi Jewish	1 in 9
East Asian	1 in 528
Finnish	1 in 7
European (Non-Finnish)	1 in 13
Native American	1 in 36
South Asian	1 in 18

Neuronal Ceroid-Lipofuscinosis (CLN3-Related) (CNL3 gene) carrier status frequency in different ethnicities, from Sema4's website:

Worldwide	1 in 434

African	1 in 1697
East Asian	1 in 589
Finnish	1 in 1722
European (Non-Finnish)	1 in 242
Native American	1 in 1538
South Asian	1 in 2552

Ornithine Aminotransferase Deficiency (OAT gene) carrier status frequency in different ethnicities, from Sema4's website:

Worldwide	1 in 595
African	1 in 2898
Ashkenazi Jewish	1 in 614
Finnish	1 in 138
European (Non-Finnish)	1 in 749
Native American	1 in 1291
South Asian	1 in 905

Recommendation: Both Sema4 and Cryobio recommend that the recipient, or egg source if different than recipient, be tested for Gitelman syndrome (SLC12A3 gene, and consider CKCNB gene), nephrotic syndrome (NPHS2-Related)/steroid-resistant nephrotic syndrome (NPHS2 gene), neuronal ceroid-lipofuscinosis (CLN3-Related) (CLN3 gene), ornithine aminotransferase deficiency (OAT gene) carrier status and consider genetic counseling. Please refer to Sema4's website, sema4.com, for more information and contact Cryobio with any questions or to arrange genetic counseling. Because the donor was tested by Sema4, Cryobio recommends that the recipient or egg source should be tested by Sema4 as well. We also strongly recommend that you discuss the donor's genetic carrier status results with your health care provider. Finally, we recommend that any future child be notified of this donor's carrier status once they are of reproductive age, as even if they do not have a recessive disease, they could be a carrier and their carrier status could be important to identify risks related to their own reproductive future.

Cryobio has advised us of the following:	Please initial to show your understanding and agreement:
The donor we have chosen has had positive results from genetic testing. These results indicate that the donor is a carrier for Gitelman syndrome, nephrotic syndrome (NPHS2-Related)/steroid-resistant nephrotic syndrome, neuronal ceroid-lipofuscinosis (CLN3-Related), ornithine aminotransferase deficiency.	Initials: _____ Initials: _____
These genetic conditions are inherited as recessive traits. This means that if both the egg source and the sperm source are carriers for the same condition, there is a significantly higher chance of the resulting child having that genetic condition.	Initials: _____ Initials: _____
By the donor testing positive for carrier status for Gitelman syndrome, nephrotic syndrome (NPHS2-Related)/steroid-resistant nephrotic syndrome, neuronal ceroid-lipofuscinosis (CLN3-Related), ornithine aminotransferase deficiency the risk to a resulting child would now be higher than that of the general population.	Initials: _____ Initials: _____

Both the risk of being a carrier and the sensitivity of the genetic testing can vary depending on the individual's ethnicity. When an individual tests negative for carrier status, it does not completely eliminate their chance of being a carrier for that disease. Instead, their remaining (residual) risk of being a carrier is determined by their ethnic background. While a negative result decreases the likelihood that an individual is a carrier, how much that risk is reduced by can vary significantly. For more detailed information regarding the sensitivity of testing and remaining risk after negative screening, please see Sema4's website.	Initials: _____ Initials: _____
Genetic testing looking at a large panel of genes, including the genes/conditions that the donor has tested positive for, is available and could be done.	Initials: _____ Initials: _____
Genetic testing is *strongly recommended* for me (or the egg source, if different) to see if I am a carrier for Gitelman syndrome (SLC12A3 gene and consideration of CLCKNB gene), nephrotic syndrome (NPHS2-Related)/steroid-resistant nephrotic syndrome (NPHS2 gene), neuronal ceroid-lipofuscinosis (CLN3-Related) (CLN3 gene), ornithine aminotransferase deficiency (OAT gene).	Initials: _____ Initials: _____
A negative genetic test result in the egg source significantly reduces the likelihood that the resulting child could be affected with these conditions. However, we fully understand that the risk cannot be completely eliminated.	Initials: _____ Initials: _____
While genetic testing can lower the likelihood for certain genetic diseases, no amount of genetic testing can guarantee that a child will be healthy or free of genetic disease.	Initials: _____ Initials: _____
Genetic counseling is available to us, either through Cryobio or Sema4, if we have additional questions regarding these test results and potential risks.	Initials: _____ Initials: _____
Both the donor's carrier status and whether the donor is acceptable for my use should be discussed with my health care provider.	Initials: _____ Initials: _____

We have read the above material and assume the risk of using donor sperm from a donor who has been found to be a carrier of genetic disorders. We are making the choice to use donor sperm from donor CB 493 willingly and agree to release any legal claims, including negligence, that may arise from or are related to insemination or assisted reproduction using donor sperm from donor CB 493.

We have read and had the chance to ask questions, and we understand and agree to the terms of this Additional Agreement to use donor CB 493.

_____
Recipient

_____
Date

_____
Partner, if applicable

_____
Date

William C. Baird, PhD

_____
Cryobio

04-06-22

_____
Date