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HUMAN GENETICS • REVIEW

Do GWAS and studies of heterozygotes for *NPC1* and/or *NPC2* explain why NPC disease cases are so rare?

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Abstract

Early onset Niemann-Pick C diseases are extremely rare, especially Niemann-Pick C2. Perhaps unusually for autosomal recessive diseases, heterozygotes for mutations in *NPC1* manifest many biological variations. *NPC2* deficiency has large effects on fertility. These features of NPC1 and NPC2 are reviewed in regard to possible negative selection for heterozygotes carrying null and hypomorphic alleles.

Keywords Niemann-Pick C disease · Heterozygote disadvantage · Carrier frequency · Obesity · Fertility · Dementia

Introduction

Niemann-Pick type C (NPC) disease is a very rare autosomal recessive, neurodegenerative lysosomal storage disorder with variable clinical phenotypes (Vanier 2010). The most common presentation of NPC disease is a child of either sex developing coordination problems, dysarthria, and hepatosplenomegaly during early school-age years.

Failure of upward gaze is pathognomonic. Phenotypic manifestations are accompanied by abnormal intracellular accumulation of cholesterol and glycosphingolipids in a variety of tissues, including the liver and spleen, and progressive cerebellar degeneration (Vanier and Miller 2003). The neurological progression of the disorder is relentless and characterized by an increasing severity of ataxia, dysarthria, and dementia until death occurs, usually during the second decade of life. Seizures are a common manifestation (Vanier 2010). More severe infantile forms, where liver involvement is great (Kelly et al. 1993; Erickson et al. 2005), and late-onset forms, including Niemann-Pick D disease which is due to a hypomorphic mutation in *NPC1*, also occur. *NPC1* encodes the NPC1 protein which regulates the transport of lipoprotein-derived cholesterol from late endosomes/lysosomes to other cellular compartments and is responsible for maintaining intracellular cholesterol homeostasis (Erickson 2013). While NPC disease is most commonly associated with mutations in the *NPC1* gene (Park et al. 2003), it can also be caused by mutations in a second gene, *NPC2*, which encodes a smaller, soluble lysosomal protein, which presents cholesterol to NPC1 (Naureckiene et al. 2000; Infante et al. 2008).

The frequency of autosomal recessive diseases is quite variable, from 1/10,000 cases for phenylketonuria in Germany to 1/100,000 cases of Tay-Sachs disease in non-Ashkenazi Jews (Reich and Lander 2001). Recent massively parallel human sequencing efforts have suggested much higher carrier frequencies for deleterious alleles than expected from the frequency of diagnosed cases (Bell et al. 2011; Xue et al. 2012; Piton et al. 2013), but more refined analyses suggest that many mutants classified as deleterious are not (Lek et al. 2016). The effects of the recent rapid expansion of the world's human population for the relative paucity of autosomal recessive disease have recently been reviewed (Erickson and Mitchison 2014). A modal carrier frequency of about 1/100 would lead to a disease frequency of about 1/40,000. Carrier frequencies higher than these are thought to reflect heterozygote advantage, e.g., the common allele of cystic fibrosis (delta F508) with some resistance to infantile diarrhea (Pier et al. 1998) or sickle cell disease with decreased severity of malaria (Allison 1954). There are many other factors which influence mutational load—the size of the gene influencing the number of mutations (e.g., *Neurofibromin 1*; Upadhyaya et al. 1997), the dosage sensitivity of the pathway (signaling and transcription factors versus enzymes), and stochastic processes (Cook et al.

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1998). Lower carrier frequencies would suggest heterozygote disadvantage. The frequency of severe (null or severely hypomorphic mutations) Niemann-Pick C diseases (NPC) is clearly lower than many other recessive diseases: the disease frequency was initially estimated at about 1/150,000 overall (Vanier and Miller 2003), although a more recent estimate was 1/90,000 with late onset forms (mild hypomorphs) having a frequency of about 1/25,000 (Wassif et al. 2016). Niemann-Pick D, with its young adult onset, has achieved high frequency in an inbred population in Nova Scotia (Winsor and Welch 1978). Since NPC2 represents only 5% of cases, even assuming the higher disease frequency of 1/25,000, its frequency is much lower at about 1 in half a million! I herein review many studies on the influence of seemingly benign variants and manifestations in carriers, as well as findings in disease homozygotes which might manifest to some degree in carriers, for the more severe mutations (both man and mice)—those which might indicate selective disadvantages.

Possible causes of negative selection for carriers of detrimental mutations in the *NPC1* gene

Obesity

A large (for the time) genome-wide association (GWAS) study (Meyre et al. 2009) indicated that the Niemann-Pick C1 (*NPC1*) gene is associated with early-onset and morbid adult obesity. They studied 1380 cases of early onset or morbid obesity and 1416 controls, replicated with a study of 2100 obese and 2400 nonobese individuals and the study of 38 markers in 14,186 subjects. The association of obesity with *NPC1* variants has been confirmed in many studies (reviewed in Lamri et al. 2018). The nonsynonymous single-nucleotide polymorphism variant (rs1805081), which results in the replacement of an arginine for histidine at amino acid 215 (H215R), is associated with obesity. It is not known how this variant affects the tendency to obesity but rare loss of function variants of *NPC1* have been associated with obesity (Liu et al. 2017). Using a mouse model, it was found that decreased *Npc1* gene dosage is associated with weight gain in mice fed a high-fat diet, consistent with a gene–diet interaction (Jelinek et al. 2010). Although speculative, decreased *Npc1* gene dosage in the presence of a high-fat diet may lead to an inability to maintain normal energy and metabolic homeostasis (also reviewed in Lamri et al. 2018). It is clear that not only diet but genetic background effects have a great influence on the effect of decreased levels of the Npc1 protein (Borbon et al. 2012) which makes it hard to generalize the mouse results to humans.

There is a long history of finding metabolic alterations in human heterozygotes for *NPC1* which might be relevant to the influence on obesity. Studies in fibroblasts from patients, carriers, and controls demonstrated lower level of cholesterol esterification in the patient's cells while heterozygotes had a lower initial rate of esterification compared to normal (Kruth et al. 1986). (The same result was found with heterozygous fibroblasts in the feline model [Brown et al. 1996].) Caveolin 1, which deficiency is associated with a lean phenotype (Razani et al. 2002) is greatly elevated in heterozygotes both in man and mice (Garver et al. 1997a, b). Metabolic profiling of plasma samples by nuclear magnetic resonance analysis distinguished samples from *NPC1* carriers from healthy controls on the basis of lipoprotein triacylglycerols signals which also indicates an effect on intermediary metabolism (Probert et al. 2017). Heterozygous carriers of severe mutations had a 4.8-fold risk of morbid obesity. Severe obesity decreases both male and female fertility (Cabler et al. 2017). However, it is also hard to know if an influence of *NPC1* mutations on weight gain would be a strong selective force—most of human evolution has occurred with limited food intake and only severe obesity influences fertility.

The immune system, inflammation, and atherosclerosis

There have been many studies on the effects of loss of NPC1 on immune function. The major finding is that there is a decrease in certain classes of natural killer cells (Sagiv et al. 2006) and their distribution is altered (Speak et al. 2014) which affects antigen presentation. Also, NPC1 has been strongly implicated as a virus receptor. It is the essential receptor for Ebola virus (Carette et al. 2011) and is needed for Ebola virus replication—in the $Npc1^{-/-}$ model, early, high levels of virus are cleared and the mice remain well (Herbert et al. 2015). On the other hand, enterovirus replication was greatly enhanced in NPC1 or 2 deficient human fibroblasts (Ilnytska et al. 2013). A role of NPC1 and 2 proteins in host:symbiont interaction can be traced far back in evolution—they are important in cnidarians (corals and sea anemones; Dani et al. 2014). To my knowledge, alterations in these pathways have not been studied or found in heterozygotes and their role in selection is unclear.

On the other hand, very strong effects on inflammatory processes have been found in *NPC* heterozygotes. Macrophages are greatly affected by their cholesterol content. Free cholesterol in the cell markedly inhibits efflux of cholesterol and the partial loss of NPC1 in heterozygous cells markedly abrogates this inhibition (Feng and Tabas 2002). The mechanism affected by the decreased dosage occurs at the endoplasmic reticulum and involves the unfolded protein response (Feng et al. 2003a). This heterozygous defect would affect responses to many pathogens but was beneficial in a

mouse model of atherosclerosis with decreased necrosis in atherosclerotic lesions (Feng et al. 2003b). Thus, a decreased amount of NPC1 might have been detrimental in the past with higher disease burden but could now be beneficial to carriers.

Finally, platelet dysfunction has been described in several severe cases and confirmed in a zebrafish model (Louwette et al. 2013). Interestingly, the three patients described with a platelet granule secretion defect all had the infantile form of the disease. The zebrafish model showed thrombocytopenia and mild anemia (Louwette et al. 2013) Again, whether reduced levels of NPC1 as would be found in carriers has an effect on bleeding time is unknown.

Sporadic, late onset Alzheimer's disease

Niemann-Pick C disease has often been called juvenile Alzheimer's on the basis of the dementia which develops and the presence of neurofibrillary tangles (see below). Both disorders are greatly influenced by cholesterol metabolism (Fiorenza et al. 2013). The possibility that alterations in intracellular cholesterol transport would affect the pathological processes of sporadic, late onset Alzheimer's disease (SLAD) has been tested in vitro and in the mouse model of NPC1, *Npc1*$^{-/-}$. Increased intracellular cholesterol levels were found to be associated with increased Aβ42 in endosomes in in vitro models of NPC1 (likely to be in endosomes or lipid rafts as it was measured in the Triton insoluble fraction [Yamazaki et al. 2001]), and amyloid precursor protein (APP) is shifted to lipid rafts (Kosicek et al. 2010) but decreased on the cell surface (Malnar et al. 2010). This increase has been confirmed in vivo, in the *Npc1*$^{-/-}$ brain, and it has been demonstrated that it occurs because of a redistribution of presenilin 1, an enhancement of γ-secretase activity and an accumulation of C-terminal amyloid fragments (Burns et al. 2003). An extension of these findings implicated the early endosomal compartment (rab5-positive) more than the cholesterol-laden late endosomes (Jin et al. 2004). Importantly, increased levels of Aβ42 were found in Purkinje cells, the first neurons to die in NPC1. Perhaps surprisingly, *Npc1*$^{-/-}$ mice without amyloid precursor (Nunes et al. 2011), similar to mice without tau (Pacheco et al. 2009), show more severe pathology and shortened life expectancy.

There is now genetic evidence of interaction between NPC1 and SLAD. Single-nucleotide polymorphisms (SNPs) in *NPC1*, using centenarians as an additional control, have been implicated in the incidence of SLAD (Erickson et al. 2008). (Centenarians may have dementia but almost never have Alzheimer's; age-matched controls without Alzheimer's are still at risk.) The association of SLAD with *NPC1* was confirmed in a Han population (with only age-similar controls but with opposite risks for the rs1788799 G allele; i.e., in this population, it was a low risk, instead of high risk allele; Yang et al. 2013). The influence of *NPC1* variation on SLAD was found to be dependent on variation in *ABCA1* in a study of Spaniards (which also did not have centenarians as an additional control; Rodriguez-Rodriguez et al. 2010). In a study of 50 demented patients with corticobulbar or corticobasal symptoms, 4 were carriers of harmful mutations in *NPC1* or *2*, further suggesting the possibility of a role of NPC in other dementias (Cupidi et al. 2017). A single patient with a heterozygous mutation presented with tremor (Josephs et al. 2004).

Npc1$^{+/-}$ mice crossed with APP/PS1 "Alzheimer's" mice were studied for Aβ42 accumulation and amyloid plaque formation (Borbon and Erickson 2011). Mice heterozygous for *Npc1*$^{-}$ and positive for the APP and PS1 transgenes accumulated Aβ42 more rapidly than the APP/PS1 controls and this correlated, as expected, with the area of amyloid plaques (Borbon and Erickson 2011). Thus, alterations of intracellular cholesterol present in *Npc1*$^{+/-}$ mice influence the progress of Alzheimer's disease in the APP/PS1 mouse model, a result confirmed by Maulik et al. (2013).

There have been several studies of neurological manifestations found in mouse heterozygotes for the null *Npc1*^{nih} mutation. Partial motor dysfunction and anxiety-like behavior was found at 9 weeks of age by Hung et al. (2016). At 2 years of age, increased brain cholesterol, Purkinje cell loss, and altered tau phosphorylation were found in the heterozygotes but not the wild-type controls (Yu et al. 2005). Whether human heterozygotes would have such problems and whether they would affect fecundity is unknown.

While a role for variants in *NPC1* in the onset of SLAD seems established, it is not clear whether this relationship is due to decrease NPC1 levels and, hence, detrimental to carriers. Since SLAD is a late onset disease, its occurrence is unlikely to provide much of a negative selection force.

Effects on reproduction

Altered fertility can exert a strong influence on gene frequencies. One feature of *Npc1*$^{-/-}$ mice is their infertility. Most of the studies have been performed on this null mutation. The model of juvenile Npc1, a point mutation with greatly decreased protein (*Npc1*^{nmf164}; Maue et al. 2012), is fertile but the mice usually only have one litter—the rapidly advancing disease soon prevents breeding.

Fertility is dependent on the generation and continuous production of sex hormones, which are essential for physiological regulation. Cholesterol serves as a precursor for steroid hormone synthesis. Cholesterol for this synthesis can be obtained in three different ways: through de novo synthesis, through endocytosis of low-density lipoproteins (LDL), and through the selective uptake of cholesterol through high-density lipoproteins (Gwynne and Strauss 1982). The mutation in *Npc1* causes cholesterol derived from low-density lipoproteins to build up in the cell, but the other two sources of

cholesterol also provide the sterol needed for hormone synthesis. A decrease in testosterone levels has been multiply reported (Roff et al. 1993; Akpovi et al. 2014). However, the lack of Npc1 was not found to decrease available levels of cholesterol substrate for steroid hormone synthesis (Xie et al. 2006), creating a conundrum.

There have been multiple investigations as to the cause of female infertility in the *Npc1*$^{-/-}$ mouse. Erickson et al. (2002) found that breeding *Npc1*$^{-/-}$ doubly homozygous recessive with the *mdr1a*$^{-/-}$ (multiple drug resistance 1a knockout) mice restored the lacking *Npc1*$^{-/-}$ female fertility. This was interpreted as increasing cholesterol transport to the endoplasmic reticulum and providing this precursor for estrogen and progesterone synthesis. However, *Npc1*$^{-/-}$ pre-treated with pregnant mare serum (PMS) and human chorionic gonadotrophin (hCG) showed normal numbers of corpora lutea and levels of progesterone suggesting a deficiency of pituitary hormones (Erickson et al. 2002). This was confirmed by Gevry et al. (2006). This group reported a marked increase in the dopamine D2 receptor (DRD2) in *Npc1*$^{-/-}$ pituitaries. They concluded that the *Npc1* mutation affects the ovarian-pituitary-hypothalamic feedback loop by interfering with estrogen production, and demonstrated that chronic treatment with estrogen in the *Npc1*$^{-/-}$ mouse restored prolactin expression (Gevry et al. 2006).

Zhang et al. (2008) found that controlled expression of the NPC1 protein with directed synthesis of Npc1 in astrocytes, using the glial fibrillary acidic protein (GFAP) promoter, lowered cholesterol storage in neurons and corrected fertility. Low acidophil (cells producing LH and FSH) counts in the pituitaries of *Npc1*$^{-/-}$ were corrected in the transgenic mice which express the Npc1 protein exclusively in fibrillary astrocytes (Donohue et al. 2009), supporting the notion that this sterility was of hypothalamic origin.

Male fertility has also been extensively studied. Erickson et al. (2002) found that male sterility in *Npc1*$^{-/-}$ mice could be corrected merely by changing the genetic background, i.e., moving the mutation onto a different inbred strain. Fan et al. (2006) found decreased binding of sperm to the zona pellucida (ZP) and decreased proteolytic processing of cyritestin, a sperm surface protein essential for binding to the ZP. The correct cholesterol content is crucial for normal sperm function (Sugkraroek et al. 1991). Indeed, high cholesterol levels in sperm are negatively correlated with sperm quality in humans (Zalata et al. 2010). Moreover, cholesterol may have an impact on spermatozoal membrane lipid raft function (Cross 2004). Studies in isolated seminiferous tubules of *Npc1*$^{-/-}$ mice showed elevated free and esterified cholesterol and cholesterol regulatory proteins but serum testosterone was markedly low (Akpovi et al. 2014). This study found disorganized spermatogenesis, an increase in both the number of apostatin-positive cells and the tubular apoptosis levels measured by ELISA, and elevated Fas and FasL levels (tumor necrosis family ligands which induce apoptosis on binding their receptors).

Whether any of these abnormalities related to male and female infertility are also present in heterozygotes has not been determined—the heterozygous mice have normal litter size but produce a lower-than-expected number of homozygotes. This loss (~17% instead of the 25% expected) is presumably post-zygotic. I am not aware of studies on human heterozygous fertility. Thus, the possible influence of factors relating to fertility on gene frequency of deleterious alleles of *NPC1* is unknown at present.

Possible causes of negative selection for carriers of detrimental mutations in the *NPC2* gene

The gene in which mutations cause NPC2 disease was identified by Naureckiene et al. (2000) and had been first cloned as the gene encoding a major soluble protein in semen, HE1 (Kirchoff et al. 1996). Although frequently considered merely as a donor of cholesterol to NPC1 (Subramanian and Balch 2008), it has both different intracellular (Zhang et al. 2003) and tissue localizations. NPC2 plays a separate role from that of NPC1 in cholesterol export (Boadu et al. 2012), and its potential role in neurodegeneration may differ from that of NPC1 as it is predominantly expressed in neurons while NPC1 is predominantly expressed in astrocytes (Ong et al. 2004). It is an abundant secretory protein in bile (Klein et al. 2006) and its overexpression promotes gallstone formation (Acuna et al. 2016). NPC2, or its orthologues in other mammalian species, is found in milk (Larsen et al. 1997) and plasma (de Arujo et al. 2016). Like NPC1, it is implicated in obesity (Csepeggi et al. 2010; Hannaford et al. 2012), intriguingly involving a membrane cell adhesion molecule characterized as a neuronal growth regulator (NEGR1; Kim et al. 2017). It, too, influences the immune system, by its effects in macrophages (Hannaford et al. 2012) and, possibly, by having a role in innate immunity, as indicated by its homolog's role in *Drosophila* (Shi et al. 2012). Finally, it has been implicated in salt transport in the kidney (Araki et al. 2009). Despite these differences in function from NPC1, the disease its deficiency causes is nearly identical to that caused by deficiency of NPC1, primarily differing in the severity of lung involvement.

NPC2 makes up 20% of the protein in chimpanzee seminal fluid (Fröhlich and Young 1996) and is thought to be important for sperm maturation (Turner 1995). The sperm of homozygous knockout mice have deficient cholesterol and reduced in vitro fertilizing capacity (Busso et al. 2013), while females have anovulation and decreased progesterone (Busso et al. 2009). A specific role has been found for the porcine orthologue in resistance to freezing (Valencia et al. 2017). Thus, it is quite probable that *NPC2* carriers may express lower levels in seminal plasma and have decreased fertility. While the alterations related to NPC1 function in sperm are

intracellular, NPC2 may affect these as well as having an important role in semen as indicated by its high concentration there. Again, these parameters of fertility have not been studied, to my knowledge, in either human or animal heterozygotes, so a role in affecting gene frequency is speculative.

Conclusion

Although there are a large number of changes in the physiology of *NPC1* and *NPC2* heterozygotes, both in man and mice, their effects on carrier gene frequencies is speculative. Severe obesity decreases fertility and this may be a factor influencing *NPC1* carrier frequency. However, as noted, most of human evolution has occurred under conditions of food scarcity. The many studies on the role of NPC2 in semen and spermatozoa suggest the possibility that decreased fertility in carriers of damaging mutations in *NPC2* might result but, as yet, there is no evidence for this in man or mice. However, this possibility remains the best available explanation for the extremely low gene frequency of damaging alleles of *NPC2*.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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