Niemann-Pick Disease:
A Synopsis of the Genetic Variation Among Various Types

Joanna Hays

Prof. John Hnida
Peru State College
April 19, 2006

Overview

Niemann-Pick Disease (NP) occurs in patients with deficient acid sphingomyelinase (ASM) activity, as well as the lysosomal accumulation of sphingomyelin. It is an autosomal recessive disorder (Levran et al. 1991). As recently as 1991, researchers had classified two major phenotypes; Type A and Type B (Levran et al. 1991). In more recent studies several more phenotypes have been identified, including Types C and D. Each type of NP has distinct characteristics and effects on the patient. NP is distributed worldwide, but is closely associated with Ashkenazi Jewish descendants. Niemann-Pick Disease is relevant to the molecular world today because of advances being made in the ability to identify mutations, to trace ancestry where the mutation may have originated, and to counsel patients with high potential of carrying the disease. Genetic counseling primarily consists of confirmation of the particular disease and calculation of the possible future reappearance in the same gene line (Brock 1974). The following discussion will summarize the identification of mutations causing the various forms of NP, the distribution of NP, as well as new genotypes and phenotypes that are correlated with NP.

Mutations causing NP

Levran et al. (1991) informs readers of the frequent identification of missense mutations in the gene associated with Ashkenazi Jewish persons afflicted by Type A and Type B NP. This paper identifies the mutations associated with NP and the beginning of many molecular techniques to develop diagnoses. Greer et al. (1998) identifies a new mutation that is specifically identified to be the cause of Type D. NP in various forms is closely associated with the founder effect caused by a couple married in the early 1700s in what is now Nova Scotia. Simonaro et al. (2002) discusses the distribution of Type B NP as well as new phenotypes and genotypes. All three of these papers identify the correlation of NP with the Ashkenazi Jews, but also find discontinuity among the different forms of NP and the Ashkenazi Jews. Each paper also urges the importance of the ability of genetic counseling to prevent heterozygote carriers of passing the sometimes lethal allele on to their offspring.

Type A NP (NPA) is a fatal neurodegenerative disorder. NPA causes severe psychomotor retardation and enlargement of the spleen and liver. It is identified in infancy and is lethal in the first 2–3 years of life (Levran et al. 1991). Levran et al. distinguishes the difference between NPA and Type B (NPB) persons of Ashkenazi Jewish descent, with NPA being homoallelic and NPB heteroallelic. By developing a functional cDNA region, they identified the mutation of a guanine to a thymine in nucleotide 1487. This causes an arginine to leucine substitution on polypeptide 496. This missense mutation is referred to as R496L and is frequently found in NPA and NPB people of Ashkenazi descent.

Levran et al. (1991)

Levran et al. performed various laboratory experiments to identify the missense mutation, consisting of the development of cell lines, enzyme assays, gene amplifications, and dot-blot analysis. Fibroblast cultures and blood samples were taken from NP patients and normal patients. To identify NPA or NPB, a significant decrease in ASM activity must have occurred in the cultured cells. A protein assay was done on the cultured cells. Reverse transcription was done on the cDNA amplification by polymerase chain reaction (PCR). Dot-blot analysis was performed after the amplified cDNA was run in gel electrophoresis.

R496L mutation was confirmed to be a real homoallelic mutation in the patient of Ashkenazi Jewish descent, by comparison of his genetic material to family members and non-affected Ashkenazi Jews. This allele was not found to be present in the 180 other alleles from the normal population. This proves that the guanine to thymine change is not a common polymorphism within the lineage and in fact represents a missense mutation. R496L was prevalent in 32% of NPA Ashkenazis and 25% NPB Ashkenazis, less than 10% in NPA non-Jewish and 0% NPB non-Jewish.

Levran et al. identified three autosomal recessive disorders associated with Ashkenazi Jews: Tay-Sachs disease, Gaucher disease, and Niemann-Pick disease. All three of these disorders are associated with enzymatic defects in the sphingolipid degradative pathway. Because of this correlation, they argue the idea of founder's effect,

genetic drift, or possible convergent evolution. Their final persuasion is for the advancement of genetic counseling or molecular screening because of the high prevalence in the Ashkenazi Jewish population.

This paper, though slightly dated, gives the reader a good background on Niemann-Pick disease and is very informative on the topic of ASM activity and how it helps to determine diagnosis. However, there should have been more emphasis on the sample size and results.

Greer et al. (1998)

Greer et al. describes a different form of NP, as the Nova Scotia form or type D (NPD). Like NPA, NPD is usually fatal early in life. Patients affected by type D experience progressive neuro-degeneration and die within the first 10–20 years. The Greer paper also identifies Niemann-Pick disease as two types, each with subtypes. In this case NPA and NPB are subtypes of Type I and NPC and NPD are subtypes of Type II. This paper identifies the origination of NPD from the marriage of a couple in or near Nova Scotia around 1700, and readily refers to the disease caused by founder effect. Apparently NPD is found only in descendents of this couple. The main genetic flaw of NPD that correlates it with NPC is found on the 18q11-12 chromosome, and NPC1 is mutated in these patients. NPC1 is associated with the movement and control of cholesterol intracellularly.

Like the Levran researchers, Greer used fibroblast and blood samples to run genetic analyses on the patients. Isolations and amplifications of cDNA sequences were made. They were then reverse transcribed to identify carriers versus non-carriers. Clones of the carrier region were created and sequenced. In order to find out if the isolated gene product is responsible for NPD, the NPC1 cDNA from NPD patients was cloned. This identified a mutation of guanine to thymine at the 3097 nucleotide. This codes for a glycine to tryptophan switch on polypeptide 992. This mutation was found eight times in affected patients and zero times in unaffected patients. Forward and reverse primers were used to create gene fragments of the NPD mutation. All affected individuals were found to be homozygous for the mutation except for one. The mother of the heterozygote was the only patient

screened to not be identified as heterozygous for the mutation. None (0/50) of the unaffected patients tested for the mutation.

Once again this paper encourages the development of testing carriers of descendants as well as warning these people of the risk factors involved with genetic homozygousity. This paper differentiated between Type I and Type II Niemann-Pick disease and explained the procedure concisely with an emphasis on the results. Greer et al. also used a large study group, which proved the study had statistical importance.

Simonaro et al. (2002)

The paper by Simonaro et al. describes the worldwide distribution as "panethnic." The results of their study showed that NPB affects persons of Ashkenazi Jewish extract at much lower percentages than the more lethal Type A form of NP. NPB is almost always non-neurological and patients often survive into their adult life. NPB also differs from NPA in the fact that it is heterogenous. The pathology of NPB is still quite severe, including hepatosplenomegaly, slow growth and development, respiratory infections, and many items pertaining to hematopoeitic tissue, especially dealing with cholesterol levels. This is a much more recent publication and cites 23 mutations involving NPA or NPB. ΔR608 is identified as the first mutation associated with an NPB patient. This is caused by the absence of 3 base-pairs from exon 6 and foretold the extraction of an arginine residue from the 608 polypeptide. Unlike NPD, type B is found more frequently in North African descendants from the Maghreb region. Simonaro (2002) sampled 394 people with NPB. Blood was once again used for DNA analysis to isolate and identify the mutation.

In order to confirm NP and the SMPD1 mutation, the mutation on the acid sphingomyelinase gene, or reduced ASM activity at $<5\%$ of normal in leukocytes or fibroblasts must have been flagged in the cultured DNA. To eliminate the possibility of NPA, the patient had to be more than three years of age. By doing this, they eliminated patients not affected by NPB from the study. The distribution of the 394 patient studies was worldwide, ranging from the Americas to the Middle East and Western Europe. They found that the most common ethnic group among the patients was Turkish. They identified many

mutations among the different ethnic groups, leading to the discovery of new genotypes and phenotypes. They correlated mutations L137P, A196P, and R474W with the less severe pathology, and H421Y and K576N with early onset of NPB leading to childhood death.

In conclusion to this study, Simonaro et al. showed the worldwide distribution of NPB among many populations with different ethnic backgrounds, not limiting it to Ashkenazi Jewish descendants. Only five of the patients studied here were of Ashkenazi descent. The five Jewish patients studied did carry the ΔR608 mutation as well as one of three mutations that are closely associated with NPA. Because NPB is a heterogenous mutation, it is harder to diagnose. Many times diagnosis was not immediate and the mutation was sometimes misdiagnosed as Gaucher disease or NPC. Once again, this paper urges the use of genetic counseling for persons of extract from the ethnic groups that proved to be more frequently afflicted with NPB.

This paper was a moderately easy read. Tables and charts followed the content of the text. Some of the statistics were overwhelming, and the placement of the statistics, which were spread out throughout the results section, made them more difficult to interpret. The authors did do extensive work to eliminate other forms of NP, which none of the other papers had done.

Conclusion

Levran, Greer, and Simonaro all presented scholarly work demonstrating great success in the advancements of the study of this fascinating disease. Upon my initial research I automatically assumed that Niemann-Pick was closely associated with the Ashkenazi Jews. All of the early papers on this disease present Niemann-Pick in close association with Gaucher and Tay-Sachs diseases, all of which are directly identified among Ashkenazi Jewish populations. I chose the Levran paper because of the identification with this ethnic background. But the second and third papers under review discussed different types being closely associated with different backgrounds. Most often the authors referred to founder's effect as the cause of this disease. The unique thing about this disease is that the genetic demography is so well traced. In NPC, for example, they traced the heredity back almost to 1700, originating with a single couple; NPB is

associated with the Maghreb region and of course NPA with Ashkenazi Jews.

Overall, the discussions within these papers featured good, interesting, hard facts. Clear and concise content was presented well, even for a reader who is not an expert on molecular work. Niemann-Pick disease is very complicated. When it is broken down into types and even subtypes the fuzziness fades. I enjoyed learning of this interesting disease and the advancements that have been made in identifying the mutations, the ethnic distribution, and the urge for genetic counseling to be sought for individuals at high risk.

References

1. Brock DJH. 1974. Prenatal diagnosis and genetic counseling. J Clin Pathol Suppl. (R Coll Path.)8:150-155.

2. Greer WL, Ridell DC, Gillan TL, Girouard GS, Sparrow SM, Byers DM, Dobson MJ, Neumann PE. 1998. The Nova Scotia (type D) form of Niemann-Pick disease is caused by a $G_{3097} \rightarrow T$ transversion in NPC1. Am J Hum Genet 63:52-54.

3. Levran O, Desnick RJ, Schuchman EH. 1991. Niemann-Pick disease: a frequent missense mutation in the acid sphingomyelinase gene of Ashkenazi Jewish type A and B patients. P Natl Acad Sci USA 88:3748-3752.

4. Simonaro CM, Desnick RJ, McGovern MM, Wasserstein MP, Schuchman EH. 2002. The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. Am J Hum Genet 71:1413-1419.