Metachromatic Leukodystrophy: the Role of Non-Pathogenic Sequence Variants in the Causation of Disease

by

John Steven Harvey, B.Sc.(Hons)

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ABSTRACT

Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder caused by a deficiency of the enzyme arylsulphatase A (ASA). ASA is responsible for the degradation of cerebroside sulphate, a sphingolipid found as a major component of myelin. A deficiency of ASA leads to the accumulation and lysosomal storage of cerebroside sulphate within nerve cells, causing progressive demyelination of the central and peripheral nervous system.

MLD is diagnosed biochemically by the measurement of residual ASA activity in cultured skin fibroblasts or blood leukocytes. Diagnosis is, however, complicated by the presence of individuals in the normal population with ASA activities in the range of MLD patients. This non-pathogenic deficiency of ASA has been termed ‘ASA pseudodeficiency’ (ASA-PD) and is commonly caused by a 90% reduction in ASA mRNA levels and hence ASA protein, leaving pseudodeficient individuals with only 10% of normal ASA activity. Approximately 1% of the normal population are homozygous for the ASA-PD allele. The existence of clinically normal individuals with only 10% of normal ASA activity suggests that most ASA enzyme that is produced is far in excess of that required for the normal hydrolysis of cerebroside sulphate. The threshold level of ASA activity which separates normal development from the development of MLD has been estimated in this thesis to be between 3% and 4% of normal.

Eight ASA mutations had been identified prior to the commencement of this project. Our MLD patient population (N=29) was screened for the presence of each of these alleles to identify a subset of 13 patients in which no known pathogenic mutations were identified. Single strand conformation polymorphism and sequence analysis identified a further nine novel putative MLD mutations within this group.
In some cases there was a clear correlation between genotype and clinical presentation, for example, a single mutation, T274M, was found to account for the presentation of six MLD patients of Lebanese descent. However, numerous patients were found to have complex ASA alleles containing multiple sequence variants. The role that these multiple alterations played in the development of MLD was unclear, particularly in view of the fact that when a number of these sequence variants were expressed in vitro, they were found to reduce ASA activity without causing MLD.

The individual expression of each ASA sequence variant and the expression of complex patient alleles has lead to a greater understanding of the role that non-pathogenic changes within the ASA gene can play in the development of MLD. Specifically this work has demonstrated that;

1) Individually non-pathogenic sequence variants in the ASA gene can cause MLD in the absence of recognised MLD mutations when they are combined on a single allele.

2) When non-pathogenic sequence variants are found in combination with mutations causing mild MLD, they can act to modify the expression of disease causing mutations and increase disease severity.

3) Non-pathogenic sequence variants can have different effects on phenotype depending on the background level of ASA activity on which they are expressed.

The definition of a role for non-pathogenic sequence variants in the causation of MLD has significant consequence for the development and presentation of other lysosomal and non-lysosomal disorders.