P-3-14

LENTIVIRAL-MEDIATED CORRECTION OF METHYLMALONYL CoA MUTASE DEFICIENT MOUSE FIBROBLASTS

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Aim: To evaluate the lentiviral vector-mediated transfer of the methylmalonyl CoA mutase gene as a treatment for methylmalonyl aciduria resulting from mutase deficiency, using fibroblast cultures from a methylmalonyl CoA mutase knockout mouse as an *in vitro* disease model. **Method**: A self-inactivating lentiviral vector expressing murine methylmalonyl CoA mutase from the elongation factor 1α promoter was constructed and used to transduce cultures of MMA CoA mutase knockout mouse fibroblasts. C^{14} propionate incorporation into TCA precipitable material was measured *in vitro* by a modified version of the method of Peters et al. Vector copy number was measured by real time PCR using a transferrin gene sequence as a single copy (diploid) control. **Results**: MMA fibroblasts were efficiently transduced with the vector and this resulted in the correction of the metabolic deficiency, as indicated by propionate incorporation into TCA precipitable material. **Conclusion**: These results show that lentiviral mediated transfer of the methylmalonyl CoA mutase gene is able to correct the defect in methylmalonyl CoA mutase deficient MMA cells, suggesting that the use of lentiviral vectors expressing MMA CoA mutase may be a viable approach for the development of a 'metabolic sink' gene therapy strategy for this form of MMA.

P-3-17

CLINICAL HETEROGENEITY OF HMG-CoA LYASE DEFICIENCY

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3-Hydroxy-3-methylglutaric aciduria is a rare autosomal recessive disorder of leucine metabolism classically presenting as Reye-like illness in infancy. We have identified 3 patients with HMG CoA lyase deficiency with different presentation over past 6 years. All of them had a typical urine organic acid GC-MS profile revealing large peaks of 3-OH-3-methyl-glutaric acid, 3-methylglutaconic acid, 3-methyl-crotonylglycine and 3-OH-isovaleric acid as well as dicarboxylic acids C6>C8>C10. Serum amino acid revealed hyperglycinemia and high glutamine. The first patient was a 19 year-old girl diagnosed at the age of 13 when she presented with epileptic encephalopathy and left hemipareisis due to a right parietal infarct after a bout of diarrhoea. The other two patients are siblings from the same family with consanguineous parents. The elder sister was diagnosed at 6 months of age when she presented with cyclical vomiting and failure to thrive associated with metabolic acidosis and rasied anion gap. Mother noted abnormal odour in her urine during each attacks and she had an episode of hypoketotic hypoglycemia in the neonatal period which she recovered without further investigation. Her younger brother was diagnosed at day 3 of life with hypoketotic hypoglycemia, metabolic acidosis with increased anion gap and hyperammonemia of 900 μmol/L requring peritoneal dialysis and ventilation. Both of them had the classical presentation requiring carnitine and leucine restricted diet before they improved. The first patient with atypical presentation required no specific therapy except for avoidance of fasting particularly during intercurrent illnesses. All of them remained well to date without significant neurological deficits.

P-3-18

AMINOACID FOLLOW-UP DURING SUCCESSFUL PREGNANCY IN A WOMAN WITH METHYLMALONIC ACIDAEMIA

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There are few reports about pregnancy occurring in methylmalonic acidaemia (MMA). We report a new case of a 23 years old woman diagnosed for MMA in the neonatal period. The diagnosis was made biochemically and subsequent enzyme studies determined the complementation group to be mut^-. Metabolic control during childhood and adolescence was good and she achieved normal secondary school. Pregnancy was followed weekly from the fourth week of pregnancy. Feeding difficulties (vomiting) occurred during the first trimester and improved from the second trimester to the end of the pregnancy. The weight remained stable until the 16th week of gestation and then increased regularly. Treatment included low protein diet (20–30 g of natural protein + 80 g of protein substitute without Val, Ile, Met and Thre), carnitine, cobalamine, iron and folate supplementation, and metronidazole. She was treated in the third trimester for a moderate hypothyroidism. Urinary methylmalonic acid excretion decreased after the first trimester and remained roughly stable afterwards. Delivery occurred by caesarean section, with a normal newborn boy (weight: 3.42 kg, height: 49 cm, head circumference: 35 cm). We followed glycine as a marker of metabolic decompensation and the sum of branched chain aminoacids (BCAA) as a marker of adequate natural protein intake. Glycine increased during the first trimester (vomiting), and then completely normalised while BCAA decreased (foetal growth and liver maturation). We suggest that serum aminoacid determination (glycine and BCAA) is a reliable tools to follow the metabolic and nutritional status during pregnancy in MMA.

P-3-19

IDENTIFICATION OF MMACHC GENE MUTATIONS IN NORTHERN CHINESE cBLC PATIENTS WITH COMBINED METHYLMALONIC ACIDEMIA AND HOMOCYSTINURIA

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The *cblC* type methylmaonic acidemia (*cblC* MMA, OMIM 277400), which is caused by defects in the *MMACHC* gene, is the most frequent inborn error of vitamin B_{12} metabolism. The defect impairs the biosynthesis of adenosylcobalamin and methylcobalamin, leading to dysfunction of methylmalonyl CoA mutase and methionine syntase. Accordingly, patients carrying *MMACHC* mutations will present clinical symptoms of combined methylmalonic acidemia and homocystinuria. In this study, the ORF region of exon 1 to 4 of the *MMACHC* gene was PCR amplified and sequenced subsequently to identify molecular defects in 25 unrelated Northern Chinese *cblC* MMA families. Thirteen different alterations, which accounted for 80% of diseased alleles, were identified. These mutations included 8 point mutations (c.1A>G, c.315C>G, c.365A>T, c.394C>T, c.457C>T, c.482G>A, c.615C>A, and c.609G>A), 3 insertions (c.445_446insA, c.568_569insT, and c.626_627insT) and 2 deletions (c.658_660delAAG and c.277-3_c.303del30). Among which, c.394C>T, c.457C>T, c.482G>A, and c.609G>A had been reported in other populations, while the other mutations were identified firstly in *cblC* MMA patients. The c.609G>A was found to be the most frequent mutant allele in Chinese *cblC* MMA (32%). This mutation had been reported in East Asian previously. The c.658_660delAAG (10%) and c.482G>A (8%) were found to be the second and the third frequent mutations in Chinese patients. Mutation c.315C>G, c.394C>T, c.457C>T, c.615C>A, and c.609G>A are nonsense mutation. Insertion mutations might cause frame shift. Two deletions might cause a deletion of amino acids. Most of these alterations were predicted to cause the impaiments of protein function and therefore lead to the disease.