HEPATIC-BASED INBORN ERRORS OF METABOLISM

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ABSTRACT

Inborn errors of metabolism (IEMs) are a vast, diverse, and heterogeneous set of genetic disorders. Hepatic-based IEMs are a significant cause of morbidity and mortality, and represent a common indication for liver transplantation (LTx) in the paediatric population. This review focuses on four of the most common hepatic-based IEMs where Tx, either as whole organ liver or as isolated hepatocytes, may be an option: familial amyloid polyneuropathy, Wilson's disease, alpha-1 antitrypsin deficiency, and phenylketonuria.

Keywords: Inborn errors of metabolism, inherited metabolic disorders, liver transplantation, hepatocyte transplantation, gene therapy.

INTRODUCTION

Inborn errors of metabolism (IEMs) are a vast, diverse, and heterogeneous set of genetic disorders that are caused by alterations of a specific chemical reaction in metabolism. The term was coined by Sir Archibald Garrod in 1902.[1] Although individually rare, IEMs are collectively common with an estimated incidence >1:1,000.[2] There are hundreds of different IEMs mapped to date, and the number will probably continue to grow until all variants of enzymes and transporters that specify homeostatic mechanisms in humans are identified.[3,4] IEMs are a significant cause of morbidity and mortality, especially in childhood. A vast number of key metabolic reactions occur in the liver. This review focuses on the most common hepatic-based IEMs where transplantation (Tx), either as whole organ or as isolated hepatocytes, may be an option. Based on data from the European Liver Transplant Registry, familial amyloid polyneuropathy (FAP), Wilson's disease (WD), and alpha-1 antitrypsin deficiency (A1ATD) combined accounts for ~55% of all liver transplants for IEMs (Table 1[5-13]). In phenylketonuria (PKU), due to its relative frequency, arduous management, and grave complications, hepatocyte transplantation (HTx) is being investigated.[14]

Table 1: Hepatic-based inborn errors of metabolism (IEMs) and liver transplantation in Europe from 1988-2009.

Disorder	Incidence	No. of transplants performed
Familial amyloid polyneuropathy	1:500 to 1:100,000[5,6]	1280[7]
Wilson's Disease	1:30,000 to 1:100,000[8]	812[7]
Alpha-1 antitrypsin deficiency	1:2,000 to 1:5,000[9]	542[7]
Hereditary haemochromatosis	1:200 to 1:300[10]	468[7]
Primary hyperoxaluria	1:120,000[11]	230[7]
Tyrosinaemia Type 1	1:100,000[12]	98[7]
Homozygous hypercholesterolaemia	1:1,000,000[13]	21[7]

NB: Transplants for non-hepatic-based IEMs are excluded.

FAP

FAP is caused by a mutation in the gene that encodes transthyretin (TTR), and was first described by Mario Corino de Andrade in 1952.[15] There are >100 mutations in the *TTR* gene associated with disease,[16] the most common being V30M. FAP is an autosomal dominant disease found throughout the world, but not all carriers develop the disease. For example, Northern Sweden has a high carrier frequency of the V30M mutation - 1.5% of the population - but only 5% develop symptoms before the age of 40 years. In contrast, endemic areas in Portugal have ten times lower carrier frequency (0.18%), but high penetrance (87% before the age 40).[17] Unexpectedly, homozygote carriers do not have more severe disease than heterozygotes.[17] TTR transports thyroxin and retinol in serum and cerebrospinal fluid, and is secreted by hepatocytes.[18] TTR is a significant plasma protein (approximately 25 mg/dl),[19] and has a tendency to form amyloid in essentially all vascular organs. These amyloid deposits are found, to some degree, in 25% of the population older than 80 years,[20] usually without clinical significance. In TTR V30M, a single amino acid substitution results in a structural change of the protein, causing altered metabolism and enhanced amyloid fibril formation,[21] resulting in neuro and cardiomyocyte-toxicity at a younger age.

FAP is characterised by progressive peripheral and autonomic neuropathy or cardiomyopathy in early adulthood and results in severe disability and death within 10-15 years.[22] It is caused by deposits of mutant TTR displacing normal cellular structures, resulting in impairment of organ function.[16] Clinically, FAP should be considered in patients with a progressive axonal polyneuropathy of unknown origin, especially when associated with autonomic dysfunction or cardiac manifestation. Biopsy of an affected organ may then confirm the diagnosis. Family history is of paramount importance.[23] Medical treatment options for FAP are evolving. The working hypothesis is that if one can stabilise TTR in its tetrameric form, amyloid formation may be prevented. Tafamidis, a meglumine salt, has been shown to slow the neurological deterioration in FAP,[24] and has been approved in Europe and Japan. Another strategy is inhibition of TTR synthesis on the RNA level. Again, mainly two strategies for this exist; degradation of mRNA by antisense oligonucleotides or gene silencing using small interfering RNAs. Phase I studies for both strategies have recently been completed, and Phase II/III studies are ongoing. Both strategies appear safe, and efficiently reduce the amount of circulating TTR.[25-27]

Liver transplantation (LTx) has been performed as treatment for FAP since the early 1990s. Worldwide, over 2,000 liver transplants have been registered (www.fapwtr.org), of which 1,200 have been performed in Europe alone.[5] The overall 5-year patient survival in Europe following Tx is 76%, but 100% 10-year survival has been reported by a Japanese group.[28] TTR is not hepatotoxic, meaning that the explanted liver from a FAP patient can be transplanted into another patient with terminal liver failure. The first such 'domino' procedure was performed in 1995.[29] The V30M liver will indeed continue to produce mutant TTR, but the generally slow progression of the disease may justify the use in older recipients.[30] There is mainly one drawback with LTx for FAP (aside from the necessity of immunosuppression and risks of surgery): the transplanted liver continues to produce normal TTR, which, in some patients, continues to be incorporated into existing fibril deposits, especially in the heart.[31] Combined heart-liver Tx has been performed in patients with FAP,[32] but is not likely to become a widely available treatment option.

WD

WD is caused by mutations in the gene encoding ATP7B, and was first described Samuel Alexander Kinnier Wilson in 1912.[33] There are >500 mutations associated with WD in the *ATP7B* gene (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ATP7B). WD is an autosomal recessive disorder with a prevalence of about 1:30,000 to 1:100,000,[8] but the prevalence may be considerably higher in some areas, e.g. East Asia.[34] Genotype-phenotype correlations in WD have yet to be established, as the number of homozygotes is exceedingly small, and the prevalence of compound heterozygotes is high.[35]

ATP7B is found exclusively in the hepatocyte and permits the efficient excretion of copper into the bile. Copper is an important cofactor for many proteins, but the average diet provides an abundance and the majority ends up being excreted.[36] The most common effect of mutation in *ATPB7* is protein misfolding. This misfolding causes altered metabolism, decreased stability, and loss of copper-transport activity.[37,38] The resulting copper accumulation is toxic, especially in the

liver and brain.[39] Recently, it has been shown that copper is required for tumour growth and signalling in some cancers, however, the rate of hepatobiliary malignancies in WD is very low.[40,41] WD is characterised by liver disease in the second decade, followed by neuropsychiatric disorders in the third decade, but the clinical presentation is highly heterogeneous and may include Fanconi syndrome, cardiomyopathy, osteomalacia, and anaemia.[39] The median delay in diagnosis is reported to be 2 years.[42] Clinically, a diagnostic scoring system has been developed[43] with a high sensitivity and specificity.[44] It combines clinical findings, lab results, liver biopsy, and mutation analysis. Pragmatically, the presence of low serum caeruloplasmin and higher urine copper is sufficient to conclude the diagnosis of WD in most cases.

Kayser-Fleischer rings are present in about 50% of cases of WD at diagnosis.[8] Liver biopsy is used to define liver status in cases with ambiguous biochemical parameters and to evaluate hepatic copper levels with specific stains.[45] Medical treatment of WD include copper-chelators (penicillamine or trientine) and zinc salts, with the goal to establish and maintain normal copper homeostasis. Medical treatment can generally prevent and even reverse symptoms of copper overload, at least when initiated at an early stage in the disease.[46] However, non-responders or lack of compliance to the drug treatment may result in disease progression and acute liver failure with mortality approaching 100%. Sporadically, therapeutic plasma exchange and various forms of albumin dialysis have been reported as effective techniques for rapidly reducing serum copper levels in WD crisis, delaying or even preventing the need for LTx.[47] HTx has been proven safe, and at least transciently effective, in treating other IEMs, and could conceivably be utilised as support until chelation treatment shows its effect.[48] LTx is the treatment modality of choice in most cases of WD crisis. In Europe, >800 patients with WD have been transplanted. The 5-year overall survival rate is 85%.[5]

A1ATD

A1ATD is an autosomal recessive disease caused by mutations in the *SERPINA1* gene. The disease was discovered in 1963 by Carl-Bertil Laurell.[49] In the *SERPINA1* gene there are >120 mutations identified, but the majority of patients with severe disease are homozygous for the Z mutation.[9] A1ATD has an estimated prevalence of 1:2,000 – 5,000.[9] Not all develop disease: it is estimated that 10-35% of patients with ZZ genotypes do not exhibit any clinical symptoms.[50] In an epidemiological study carried out in Sweden over 40 years, <10% of the 127 infants that were identified had clinically significant liver disease over the first four decades of life.[51,52]

A1AT is mainly synthesised by hepatocytes and to a small degree in the lungs. The physiologic serum concentration of A1AT for adults ranges from 1.0-1.7 g/l, but as an acute phase protein, it is up-regulated during inflammation, infection, cancer, and pregnancy.[53] The most important function of A1AT is inactivation of released proteolytic enzymes in the lungs. In patients with the ZZ variant, A1AT proteins have a single amino acid substitution causing structural change, accumulation in the rough endoplasmic reticulum and decreased secretion.[54] The function of A1AT is also reduced.[55] In this sense, A1ATD is similar to the amyloidoses (e.g. FAP). As a result of decreased secretion of A1AT, overt protease activity ensues in the lungs, resulting in destruction of lung matrix components, alveolar structures, and blood vessels. Injury to liver cells also occurs, but susceptibility to disease is determined by processing abilities for misfolded A1AT. Interindividual differences in this cellular machinery are thought to be responsible for the different susceptibility to chronic liver disease.[56] Higher rates of liver cancer are found in A1ATD due to hepatic inflammation and increased liver cell turnover.[57] A1ATD typically appears with chronic obstructive pulmonary disease (COPD), emphysema, and disseminated bronchiectasis, usually between the forth and the fifth decade, but earlier onset may occur, especially in smokers.[58] In younger patients there is often a long lapse before A1ATD is diagnosed,[59] as the symptoms are attributed to a more likely diagnosis of asthma. The progression to liver cirrhosis in patients with A1ATD is usually slow. However, some patients develop early end-stage disease, with the need for LTx at a young age.

Diagnosis of A1ATD is usually made by measurement of serum A1AT concentration in combination with determination of C-reactive protein (the latter to exclude ongoing inflammation), and established with genotyping. Treatment of A1ATD lung manifestations does not differ from standard treatments of COPD.[60] Substitution therapy with A1AT derived from pooled human plasma is performed in some European countries,

but robust evidence of efficacy is so far limited.[61] Recently, a Phase I/II clinical trial with an adeno-accosiated virus as vector delivering human A1AT complementary DNA (cDNA) has been completed,[62] and may usher in a new era in the treatment of A1ATD. For hepatic manifestations, the anticonvulsive drug carbamazepine is currently being evaluated in a Phase III clinical trial.[63,64] Carbamazepine has been shown to enhance autophagy and perhaps other intracellular mechanisms for degrading deposits of misfolded A1AT. For advanced liver disease, Tx is the treatment of choice. More than 500 patients have been transplanted for A1ATD in Europe, and the 5-year overall patient survival is 85%.[5]

PKU

PKU is an autosomal recessive disease caused by mutations in the phenylalanine hydroxylase (*PAH*) gene. The disease was first described by Asbjorn Folling.[65] In the *PAH* gene >500 mutations have been mapped, and most have effects on PAH activity.[66] Established genotype-phenotype correlations are emerging.[67] PKU has a prevalence of about 1:10,000 in Europe, but for some areas it is higher.[68] PAH converts phenylalanine (Phe) into tyrosine, and is found exclusively in the liver.[69] Loss of PAH activity results in increased concentrations of Phe in the blood. Phe is an essential amino acid, and its entry into the brain is mediated by the large neutral amino acid carrier L-type amino acid transporter 1 (LAT1). Two other amino acids—tyrosine (precursor of dopamine and noradrenaline) and tryptophan (precursor of serotonine), also enter the brain via the LAT1 carrier. Since they compete for the same carrier, high concentrations of Phe in the blood impairs brain uptake of tryptophan and tyrosine.[70] Accordingly, cerebral protein synthesis rate is decreased in PKU patients when concentration of Phe is high.[71] Furthermore, animal studies have shown that high concentration of Phe and its metabolites (principally phenyl lactate and phenylacetate) exert deleterious effects on markers of bioenergetics activity in neural tissue.[72] Together with the deficiency of tyrosine and its downstream products, these factors may explain the neurotoxicity in PKU.

PKU is classified by the severity of hyperphenylalaninaemia. The normal range of blood Phe concentrations is 0.8–1.8 mg/dl; concentrations above 20 mg/dl denote classic PKU.[73] Clinically, untreated PKU leads to disturbed brain development with profound retardation, microcephaly, epilepsy, and other neurologic symptoms. Most countries have a newborn screening programme, and early detection and implementation of a Phe-restricted diet widely prevents neurological symptoms. However, the diet regimen is arduous and even patients with well-controlled PKU exhibit a variety of subtle physical, cognitive, and behavioural symptoms.[67,74-76] Perhaps unsurprisingly, it has been shown that patients with PKU spend considerably more time managing their disease than patients with Type 1 diabetes.[77] Sapropterin dihydrochloride, a pharmaceutical form of the chaperone PAH cofactor tetrahydrobiopterin, lowers plasma Phe concentrations for up to half of patients with PKU.[78] Responders reportedly also experience increases in Phe tolerance[79] and increased quality of life (QoL).[80]

One patient with PKU has received a LTx for reasons unrelated to PKU, and the patient's blood Phe level normalised after transplant.[81] HTx has been performed in one patient who had poor dietary control, with temporary improvement of blood Phe levels.[82] Clinically, HTx involves isolation of hepatocytes from livers rejected for solid organ Tx, and is performed with an infusion of the cells via a portal catheter into the liver, in a manner much resembling that of islet Tx. It is minimally invasive, and generally performed under local anesthesia. In the case of PKU, the cells need only improve a single enzyme deficiency. A clinical trial with hepatocytes for PKU is currently ongoing in the United States.[14]

CONCLUSION

Even though IEMs are regarded as simple mendelian diseases, clear genotype-phenotype correlations are rarely seen.[4] It is increasingly recognised, partly through the advent of next-generation sequencing, that multiple causative alleles, modifier alleles, or both, are common.[83] Hopefully improvements in our understanding of these genetic mechanisms will result in robust methods that can identify patients needing treatment before devastating symptoms occur. Gene therapy, and cellular Tx have the potential of dramatically improving the QoL of patients suffering from IEMs in the near future. For now, improved medical treatments and whole organ LTx may increasingly be considered in hepatic based IEMs.

REFERENCES

1. Garrod AE. The incidence of alkaptonuria: a study in chemical individuality. Lancet. 1902;2:1616-20.

2. Sanderson S et al. The incidence of inherited metabolic disorders in the West Midlands, UK. Arch Dis Child. 2006;91:896-9.

3. Peltonen L, McKusick VA. Genomics and medicine. Dissecting human disease in the postgenomic era. Science. 2001;291: 1224-9.

4. Lanpher B et al. Inborn errors of metabolism: the flux from Mendelian to complex diseases. Nat Rev Genet. 2006;7:449-60.

5. Conceição I, De Carvalho M. Clinical variability in type I familial amyloid polyneuropathy (Val30Met): comparison between late- and early-onset cases in Portugal. Muscle Nerve. 2007;35:116–8.

6. Benson M, "Amyloidosis," Scriver CR et al (eds.), The metabolic and molecular bases of inherited disease (2000), McGraw-Hill: New York City, NY, pp. 5345-78.

7. Adam R et al; All contributing centers (www.eltr.org); European Liver and Intestine Transplant Association (ELITA). Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). J Hepatol. 2012;57:675–88.

8. Ala A et al. Wilson's disease. Lancet. 2007;369:397-408.

9. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. N Engl J Med. 2009;360:2749-57.

10. Merryweather-Clarke AT et al. Global prevalence of putative haemochromatosis mutations. J Med Genet. 1997;34:275–8.

11. Cochat P et al. Primary hyperoxaluria type 1: still challenging! Pediatr Nephrol. 2006;21:1075-81.

12. de Laet C et al. Recommendations for the management of tyrosinaemia type 1. Orphanet J Rare Dis. 2013;8:8.

13. Goldstein JL, Brown MS. Familial hypercholesterolemia: identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. Proc Natl Acad Sci U S A. 1973;70:2804—8.

14. Mazariegos G et al. Liver transplantation for pediatric metabolic disease. Mol Genet Metabol. 2014;111: 418-27.

15. Andrade C. A peculiar form of peripheral neuropathy; familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. Brain. 1952;75:408-27.

16. Merrill D et al. The molecular biology and clinical features of amyloid neuropathy. Muscle Nerve. 2007;36:411-23.

17. Olsson M et al. A possible role for miRNA silencing in disease phenotype variation in Swedish transthyretin V30M carriers. BMC Med Genet. 2010;11:130.

18. Holmgren G et al. Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met30). Clin Genet. 1991;40:242-6.

19. Robbins J. Thyroxine-binding proteins. Prog Clin Biol Res. 1976;5:331-55.

20. Westermark P et al. Fibril in senile systemic amyloidosis is derived from normal transthyretin. Proc Natl Acad Sci U S A. 1990;87:2843-5.

21. Hamilton JA, Benson MD. Transthyretin: a review from a structural perspective. Cell Mol Life Sci. 2001;58:1491-521.

22. Ando Y et al. Transthyretin-related familial amyloidotic polyneuropathy. Arch Neurol. 2005;62:1057-62.

23. Planté-Bordeneuve V, Said G. Familial amyloid polyneuropathy. Lancet Neurol. 2011;10:1086-97.

24. Coelho T et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology. 2012;79:785-92.

25. Ueda M, Ando Y. Recent advances in transthyretin amyloidosis therapy. Transl Neurogener. 2014;3:19.

26. Ackermann EJ et al. Clinical development of an antisense therapy for the treatment of transthyretin-associated polyneuropathy. Amyloid. 2012;19 Suppl 1:43-4.

27. Coelho T et al. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. N Engl J Med. 2013;369: 819-29.

28. Yamashita T et al. Long-term survival after liver transplantation in patients with familial amyloid polyneuropathy. Neurology. 2012;78:637-43.

29. Furtado A et al. Sequential liver transplantation. Transplant Proc. 1997;29:467-8.

30. Merrill D, Benson MD. Liver transplantation and transthyretin amyloidosis. Muscle Nerve. 2013;47: 157-62.

31. Ihse E et al. Variation in amount of wild-type transthyretin in different fibril and tissue types in ATTR amyloidosis. J Mol Med (Berl). 2011;89:171-80.

32. Nardo B et al. Combined heart and liver transplantation in four adults with familial amyloidosis: experience of a single center. Transplant Proc. 2004;36:645-7.

33. Wilson SAK. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain. 1912;34:295-509.

34. Hahn SH et al. Pilot study of mass screening for Wilson's disease in Korea. Mol Genet Metab. 2002;76:133-6.

35. Merle U et al. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut. 2007;56:115-20.

36. Roberts EA, Schilsky ML; American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008;47:2089-111.

37. Payne AS et al. Functional expression of the Wilson disease protein reveals mislocalization and impaired copper-dependent trafficking of the common H1069Q mutation. Proc Natl Acad Sci U S A. 1998;95:10854-9.

38. Tsivkovskii R et al. The role of the invariant His-1069 in folding and function of the Wilson's disease protein, the human copper-transporting ATPase ATP7B. J Biol Chem. 2003;278:13302-8.

39. Gitlin JD. Wilson disease. Gastroenterology. 2003;125:1868-77.

40. Brady DC et al. Copper is required for oncogenic BRAF signaling and tumorigenesis. Nature. 2014;509:492–6.

41. Pfeiffenberger J et al. Hepatobiliary malignancies in Wilson disease. Liver Int. 2014;doi:10.1111/liv.12727. [Epub ahead of print].

42. Prashanth LK et al. Wilson's disease: diagnostic errors and clinical implications. J Neurol Neurosurg Psychiatry. 2004;75:907-9.

43. Ferenci P et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int. 2003;23:139-42.

44. Koppikar S, Dhawan A. Evaluation of the scoring system for the diagnosis of Wilson's disease in children. Liver Int. 2005;25:680-1.

45. Ovchinsky N et al. Liver biopsy in modern clinical practice: a pediatric point-of-view. Adv Anat Pathol. 2012;19:250-62.

46. Medici V et al. Wilson disease--a practical approach to diagnosis, treatment and follow-up. Dig Liver Dis. 2007;39:601-9.

47. Reynolds HV et al. Copper removal strategies for Wilson's disease crisis in the ICU. Anaesth Intensive Care. 2014;42: 253-7.

48. Filippi C, Dhawan A. Current status of human hepatocyte transplantation and

its potential for Wilson's disease. Ann N Y Acad Sci. 2014;1315:50-5.

49. Laurell CB, Eriksson S. The electrophoretic alpha 1-globulin pattern of serum in alpha 1-antitrypsin deficiency. Scand J Clin Lab Invest. 1963;15:132-40.

50. Tobin MJ et al. Alpha 1 antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z. A survey by the British Thoracic Association. Br J Dis Chest. 1983;77:14-27.

51. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med. 1976;294:1316-21.

52. Piitulainen E et al. Alpha1-antitrypsin deficiency in 26-year-old subjects: lung, liver, and protease/protease inhibitor studies. Chest. 2005;128:2076-81.

53. Köhnlein T, Welte T. Alpha-1 antitrypsin deficiency: pathogenesis, clinical presentation, diagnosis, and treatment. Am J Med. 2008;121:3-9.

54. Purkayastha P et al. Alpha 1-antitrypsin polymerization: a fluorescence correlation spectroscopic study. Biochemistry. 2005;44:2642-9.

55. Ogushi F et al. Z-type alpha 1-antitrypsin is less competent than M1-type alpha 1-antitrypsin as an inhibitor of neutrophil elastase. J Clin Invest. 1987;80:1366-74.

56. Teckman JH et al. The proteasome participates in degradation of mutant alpha 1-antitrypsin Z in the endoplasmic reticulum of hepatoma-derived hepatocytes. J Biol Chem. 2001;276:44865-72.

57. Topic A et al. Alpha-1-antitrypsin in pathogenesis of hepatocellular carcinoma. Hepat Mon. 2012;12:e7042.

58. Brode SK et al. Alpha-1 antitrypsin deficiency: a commonly overlooked cause of lung disease. CMAJ. 2012;184:1365-71.

59. Köhnlein T et al. Diagnostic delay and clinical modifiers in alpha-1 antitrypsin deficiency. Ther Adv Respir Dis. 2010;4:279-87.

60. Fabbri LM et al. Update in chronic obstructive pulmonary disease 2005. Am J Respir Crit Care Med. 2006;173:1056-65.

61. Stockley RA et al; Alpha One International Registry (A.I.R.). Augmentation therapy for alpha-1 antitrypsin deficiency: towards a personalised approach. Orphanet J Rare Dis. 2013;8:149.

62. Chiuchiolo MJ et al. Phase I/II study of intrapleural administration of a serotype rh.10 replication-deficient adeno-associated virus gene transfer vector expressing the human α1-antitrypsin cDNA to individuals with α1-antitrypsin deficiency. Hum Gene Ther Clin Dev. 2014;25:112-33.

63. Hidvegi T et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science. 2010;329: 229-32.

64. Wang Y, Perlmutter DH. Targeting intracellular degradation pathways for treatment of liver disease caused by α1-antitrypsin deficiency. Pediatr Res. 2014;75:133-9.

65. Fölling A. Über Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. Hoppe-Seyler's Zschr Physiol Chem. 1934;227:169-81.

66. Scriver CR. The PAH gene, phenylketonuria, and a paradigm shift. Hum Mutat. 2007;28:831-45.

67. Camp KM et al. Phenylketonuria Scientific Review Conference: state of the science and future research needs. Mol Genet Metab. 2014;112:87-122.

68. Loeber JG. Neonatal screening in Europe; the situation in 2004. J Inherit Metab Dis. 2007;30:430-8.

69. Hsieh MC, Berry HK. Distribution of phenylalanine hydroxylase (EC 1.14.3.1) in liver and kidney of vertebrates. J Exp Zool. 1979;208:161-7.

70. Pietz J et al. Large neutral amino acids block phenylalanine transport into brain tissue in patients with phenylketonuria. J Clin Invest. 1999;103:1169-78.

71. Hoeksma M et al. Phenylketonuria: high plasma phenylalanine decreases cerebral protein synthesis. Mol Genet Metab. 2009;96:177-82.

72. Duarte JM et al. Metabolic disturbances in diseases with neurological involvement. Aging Dis. 2013;5:238-55.

73. Lindner M, "Treatment Of Phenylketonuria Variants: European Recommendations," Blau N (ed.), PKU and BH4: advances in phenylketonuria and tetrahydrobiopterin (2006), SPS Verlagsgesellschaft mbH: Heilbronn, pp. 180-7.

74. Waisbren SE et al. Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. Mol Genet Metab. 2007;92:63-70.

75. Waisbren SE, Azen C. Cognitive and behavioral development in maternal phenylketonuria offspring. Pediatrics. 2003;112:1544-7.

76. Christ SE et al. Executive function in early-treated phenylketonuria: profile and underlying mechanisms. Mol Genet Metab. 2010;99 Suppl 1:S22-32.

77. Eijgelshoven I et al. The time consuming nature of phenylketonuria: a cross-sectional study investigating time burden and costs of phenylketonuria in the Netherlands. Mol Genet Metab. 2013;109:237–42.

78. Utz JR et al. START, a double blind, placebo-controlled pharmacogenetic test of responsiveness to sapropterin dihydrochloride in phenylketonuria patients. Mol Genet Metab. 2012;105:193-7.

79. Singh RH, Quirk ME. Using change in plasma phenylalanine concentrations and ability to liberalize diet to classify responsiveness to tetrahydrobiopterin therapy in patients with phenylketonuria. Mol Genet Metab. 2011;104:485-91.

80. Douglas TD et al. Longitudinal quality of life analysis in a phenylketonuria cohort provided sapropterin dihydrochloride. Health Qual Life Outcomes. 2013;11:218.

81. Vajro P et al. Correction of phenylketonuria after liver transplantation in a child with cirrhosis. N Engl J Med. 1993;329:363.

82. Stéphenne X et al. Hepatocyte transplantation using the domino concept in a child with tetrabiopterin nonresponsive phenylketonuria. Cell Transplant. 2012;21:2765-70.

83. Lu JT et al. Genotype-phenotype correlation--promiscuity in the era of next-generation sequencing. N Engl J Med. 2014;371:593-6.