Variable phenotypic expression of nonsense mutation p.Thr5* in the APOE gene

Trond P. Leren *, Thea Bismo Strøm, Knut Erik Berge

Unit for Cardiac and Cardiovascular Genetics, Department of Medical Genetics, Oslo University Hospital, Oslo, Norway

ARTICLE INFO

Article history:
Received 30 August 2016
Received in revised form 19 October 2016
Accepted 19 October 2016
Available online xxx

Keywords:
Apolipoprotein E
Diagnostics
Hypercholesterolemia
Mutation
LDL receptor

ABSTRACT

Subjects with hypercholesterolemia who do not carry a mutation in the low density lipoprotein receptor gene, in the apolipoprotein B gene or in the proprotein convertase subtilisin/kexin type 9 gene, could possibly carry a mutation in the apolipoprotein E (APOE) gene. DNA from 844 unrelated hypercholesterolemic subjects who did not carry a mutation in any of the three above mentioned genes, was subjected to DNA sequencing of the APOE gene. Two subjects were found to be heterozygous for mutation p.Thr5*. This mutation which generates a stop codon in the signal peptide, is assumed to prevent the synthesis of APOE. Family studies revealed that the mutation was carried on an APOE4 allele in both families. In one of the families only those who had an APOE2 allele as the second allele, had hypercholesterolemia. These were functionally hemizygous for APOE2 and presented with a Type III hyperlipoproteinemia phenotype. However, in the second family, hypercholesterolemia was observed in the index patient who had APOE3 as the second allele, but not in four heterozygous family members who also had APOE3 as the second allele. These findings underscore that the phenotypic expression of mutations in the APOE gene is variable and that the trait exhibits reduced penetrance.

© 2016 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The low density lipoprotein receptor (LDLR) plays a key role in cholesterol metabolism by clearing atherogenic low density lipoprotein (LDL) from plasma by receptor-mediated endocytosis [1]. Disrupted LDLR-mediated endocytosis of LDL causes autosomal dominant hypercholesterolemia [1]. The underlying genetic defect may be a mutation in the LDLR gene, in the apolipoprotein B (APOB) gene or in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene [2]. Due to the increased level of plasma LDL cholesterol, subjects with autosomal dominant hypercholesterolemia have a markedly increased risk of coronary heart disease [1]. However, efficient lipid-lowering therapy is available which may normalize the levels of plasma LDL cholesterol and reduce the risk of coronary heart disease [1,3]. Thus, it is important to diagnose patients with autosomal dominant hypercholesterolemia and this can be done by molecular genetic testing.

However, a portion of subjects with hypercholesterolemia who are referred for molecular genetic testing, does not carry a mutation in the LDLR, APOB or PCSK9 genes [4]. These subjects may therefore actually not have a monogenic hypercholesterolemia or they may carry a mutation in another gene. One such gene could be the apolipoprotein E (APOE) gene where mutations p.Leu167del (c.500_502delTCC) [5–7] and p.Arg163Cys (c.487C>T) [8] recently have been found to cause autosomal dominant hypercholesterolemia.

APOE is a constituent of chylomicrons, very low density lipoprotein, intermediate density lipoprotein and high density lipoprotein and plays a role in clearing triglyceride-rich lipoproteins from plasma [9]. It is primarily synthesized by the liver. The APOE gene contains four exons and is located on the short arm of chromosome 19. It encodes a 312 residue protein which is secreted as a mature 299 residue protein after the 18 residue signal peptide has been cleaved off during translocation to the endoplasmic reticulum [10,11]. There are three major isoforms of APOE which differ at residues 112 and 158. APOE3 (Cys112, Arg158) is the most common isoform with a frequency of approximately 0.7, while the frequencies of APOE4 (Arg112, Arg158) and APOE2 (Cys112, Cys158) are approximately 0.2 and 0.1, respectively [9]. Thus, approximately 1% of subjects are homozygous for APOE2 and of these, roughly 1% develop Type III hyperlipoproteinemia which is characterized by hypercholesterolemia and hypertriglyceridemia [9]. The underlying mechanism for the role of APOE2 in Type III hyperlipoproteinemia is that APOE2 in contrast to APOE3 and APOE4, exhibits defective binding to the LDLR [12].

To further study the role of mutations in the APOE gene as a cause of autosomal dominant hypercholesterolemia, we have screened 844 unrelated hypercholesterolemic subjects for mutations in the APOE gene.
2. Material and methods

2.1. Subjects

The subjects included in this study had been referred for genetic testing with respect to autosomal dominant hypercholesterolemia as part of ordinary health care. However, no mutations had been identified in the LDLR gene or in the PCSK9 gene, and no-one carried mutation p.Arg3527Glu (c.10579C > T, ref. seq.: NM_000384.2) in the APOB gene. Subjects who also presented with hypertriglyceridemia and who had been found to be homozygous for APOE2, were excluded from the study. A total of 844 unrelated subjects were included of which 540 were females and 304 were males. The mean age was 49.6 (±12.1) years. Their mean values for total serum cholesterol, high density lipoprotein cholesterol, triglycerides before lipid-lowering therapy was started, were 10.2 (±12.6) mmol/l, 1.6 (±0.7) mmol/l and 1.8 (±1.0) mmol/l, respectively. Their mean value for LDL cholesterol calculated according to the formula of Friedewald et al. [13], was 7.4 (± 1.1) mmol/l. However, because some uncertainty may exist regarding the fasting state at the time of measurement of lipid levels, the values for triglycerides and LDL cholesterol must be interpreted with caution.

2.2. Molecular genetic screening of the APOE gene and measurement of serum APOE levels

Screening for mutations in the APOE gene was performed by using the Sanger dideoxy sequencing method of the translated exons (exons 2-4) with flanking intron sequences. A 3730xl DNA analyzer (Applied Biosystems, Foster City, CA) was used for DNA sequencing. Regarding codon and nucleotide numbering of the APOE gene, the ATG initiation codon was codon #1 and adenine of the initiation codon was nucleotide #1. The reference sequence used for nucleotide numbering of the APOE gene was NM_000041.3. The conditions and primer sequences for polymerase chain reaction and DNA sequencing are available upon request. Serum levels of APOE were determined by ELISA using Human Apolipoprotein E ELISA Kit (Abcam, Cambridge, UK).

3. Results and discussion

3.1. Identification of mutation p.Thr5*

DNA sequencing of the APOE gene in 844 unrelated hypercholesterolemia subjects identified several normal genetic variants of which the majority has been previously reported (data not shown). None of the subjects were heterozygous for mutations p.Leu167del or p.Arg163Cys and none of the subjects were homozygous for APOE2. However, two subjects were heterozygous for mutation p.Thr5* (c.15G > A) in exon 2. To our knowledge this nonsense mutation has not previously been reported. Codon 5 is within the signal peptide and mutation p.Thr5* is expected to prevent the synthesis of APOE. Both subjects heterozygous for mutation p.Thr5* presented with hypercholesterolemia (total serum cholesterol levels of 16.0 mmol/l and 11.7 mmol/l, respectively) and hypertriglyceridemia (triglyceride levels of 6.8 mmol/l and 10.0 mmol/l, respectively) (Figs. 1 and 2). The index patient (subject II.3) in Family T0657 (Fig. 1) had xanthomas and xanthelasmas, whereas the index patient (subject III.3) in Family T5044 (Fig. 2) neither had xanthomas nor xanthelasmas. To study whether mutation p.Thr5* segregated with hypercholesterolemia, available family members were tested for this mutation and had their lipid levels measured (Figs. 1 and 2).
higher total serum cholesterol levels than those who did not carry mutation p.Thr5\textsuperscript{*}. This could suggest that heterozygosity for mutation p.Thr5\textsuperscript{*} per se may have a mild effect on plasma cholesterol levels.

To further study the mechanism for hypercholesterolemia in the two mutation p.Thr5\textsuperscript{*} heterozygotes, APOE genotyping was performed based upon the DNA sequencing data. This genotyping revealed that the two mutation p.Thr5\textsuperscript{*} heterozygotes who had hypercholesterolemia (subjects II.1 and II.3), were APOE2/4 heterozygotes, whereas the three mutation p.Thr5\textsuperscript{*} heterozygotes who did not have hypercholesterolemia (subjects II.8, II.9 and III.2), were APOE4 homozygotes (Fig. 1).

From segregation analysis in this family it is evident that mutation p.Thr5\textsuperscript{*} resided on an APOE4 allele. Thus, the two hypercholesterolemic subjects carrying mutation p.Thr5\textsuperscript{*} and who were APOE2/4 heterozygotes, have no functional APOE with respect to binding to the LDLR. It was therefore surprising that this subject presented with hyperlipidemia. In this situation one could speculate that there is a common gene source for mutation p.Thr5\textsuperscript{*}.

In contrast to the index patient in Family T0657 who was an APOE2/4 heterozygote, the index patient in Family T5044 was an APOE3/4 heterozygote. The latter patient therefore has one functioning APOE allele with respect to binding to the LDLR. Being functionally hemizygous for APOE2, they are expected to present with a phenotype of Type III hyperlipoproteinemia, similar to those who are APOE2 homozygotes. This is also what was observed.

A similar finding to ours is that of Feussner et al. [14]. They identified mutation p.Thr38\textsuperscript{*} (c.114G > A) which resided on an APOE3 allele. This mutation caused Type III hyperlipoproteinemia in subjects who had APOE2 as the second allele, but not in subjects who had APOE3 as the second allele. Moreover, Dijck-Brouwer et al. [15] have reported a frame-shift mutation (c.393dupG) leading to a premature stop codon (p.Glu114Glyfs\textsuperscript{50}) which resided on an APOE3 allele. This mutation caused Type III hyperlipoproteinemia only in subjects who had APOE2 as the second allele. Furthermore, also missense mutation p.Arg154Ser (c.460C > A) has been reported to cause hyperlipidemia if the second allele was an APOE2 allele [16].

3.3. Family T0544

A total of nine family members were studied in Family T0544. Of these, seven were heterozygous for mutation p.Thr5\textsuperscript{*} (Fig. 2). However, none of these had hypercholesterolemia or hypertriglyceridemia. Segregation analysis revealed that mutation p.Thr5\textsuperscript{*} resided on an APOE4 allele also in this family. Because families T0657 and T5044 are from a restricted area in the most Southern part of Norway, it is assumed that there is a common gene source for mutation p.Thr5\textsuperscript{*}.
References

[1] J.L. Goldstein, H.H. Hobbs, M.S. Brown, Familial hypercholesterolemia, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The metabolic & molecular basis of inherited disease, McGraw-Hill, New York 2001, pp. 2863–2914.

[2] A.K. Sourat, Rare genetic causes of autosomal dominant or recessive hypercholesterolemia, IUBMB Life 62 (2010) 125–131.

[3] R.S. Patel, E.M. Scopolitti, J. Savelloni, Therapeutic management of familial hypercholesterolemia: current and emerging drug therapies, Pharmacotherapy 35 (2015) 1189–1203.

[4] M. Futema, R.A. WHittall, A. Kiley, J.A. Cooper, E. Badmuis, S. Leigh, F. Karpe, H.A. Neil, Simon Broome Register Group, S.E. Humphries, Analysis of the frequency and spectrum of mutations recognized to cause familial hypercholesterolemia in routine clinical practice in a UK specialist hospital clinic, Atherosclerosis 229 (2013) 161–168.

[5] M. Marduel, K. Ouguerram, V. Serre, D. Bonnefont-Rousselot, A. Marques-Pinheiro, K.E. Berge, M. Devillers, G. Luc, J.M. Lecerf, L. Tosoloni, D. Erlrich, G.M. Pelosi, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Averna, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.

[6] M. Rolleri, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Avena, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.

[7] M. Rolleri, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Avena, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.

[8] M. Futema, R.A. Whittall, A. Kiley, J.A. Cooper, E. Badmuis, S. Leigh, F. Karpe, H.A. Neil, Simon Broome Register Group, S.E. Humphries, Analysis of the frequency and spectrum of mutations recognized to cause familial hypercholesterolemia in routine clinical practice in a UK specialist hospital clinic, Atherosclerosis 229 (2013) 161–168.

[9] M. Marduel, K. Ouguerram, V. Serre, D. Bonnefont-Rousselot, A. Marques-Pinheiro, K.E. Berge, M. Devillers, G. Luc, J.M. Lecerf, L. Tosoloni, D. Erlrich, G.M. Pelosi, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Averna, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.

[10] M. Futema, R.A. Whittall, A. Kiley, J.A. Cooper, E. Badmuis, S. Leigh, F. Karpe, H.A. Neil, Simon Broome Register Group, S.E. Humphries, Analysis of the frequency and spectrum of mutations recognized to cause familial hypercholesterolemia in routine clinical practice in a UK specialist hospital clinic, Atherosclerosis 229 (2013) 161–168.

[11] M. Marduel, K. Ouguerram, V. Serre, D. Bonnefont-Rousselot, A. Marques-Pinheiro, K.E. Berge, M. Devillers, G. Luc, J.M. Lecerf, L. Tosoloni, D. Erlrich, G.M. Pelosi, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Averna, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.

[12] K.H. Weisgraber, T.L. Innerarity, R.W. Mahley, Abnormal lipoprotein receptor-binding activity of the human apoprotein E gene due to cysteine-arginine interchange at a single site, J. Biol. Chem. 257 (1982) 2518–2521.

[13] W.T. Friedewald, R.J. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.

[14] G. Feussner, V. Feussner, M.M. Hoffmann, J. Lohrmann, H. Wieland, M. Zörk, Molecular basis of Type III hyperlipoproteinemia in Germany, Hum. Mutat. 11 (1998) 417–423.

[15] D.A.J. Dijk-Brouwer, J.J. van Doormaal, I.P. Kema, A.M. Brugman, A.K. Kingma, F.A.J. Muskiet, Discovery and consequences of apolipoprotein-E gene mutations in the Netherlands, and mapping to chromosome 19 of the human apolipoprotein E gene, J. Lipid Res. 57 (2016) 482–491.

[16] M. Rolleri, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Avena, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.

[17] M. Futema, R.A. Whittall, A. Kiley, J.A. Cooper, E. Badmuis, S. Leigh, F. Karpe, H.A. Neil, Simon Broome Register Group, S.E. Humphries, Analysis of the frequency and spectrum of mutations recognized to cause familial hypercholesterolemia in routine clinical practice in a UK specialist hospital clinic, Atherosclerosis 229 (2013) 161–168.

[18] M. Marduel, K. Ouguerram, V. Serre, D. Bonnefont-Rousselot, A. Marques-Pinheiro, K.E. Berge, M. Devillers, G. Luc, J.M. Lecerf, L. Tosoloni, D. Erlrich, G.M. Pelosi, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Averna, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.

[19] M. Marduel, K. Ouguerram, V. Serre, D. Bonnefont-Rousselot, A. Marques-Pinheiro, K.E. Berge, M. Devillers, G. Luc, J.M. Lecerf, L. Tosoloni, D. Erlrich, G.M. Pelosi, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Averna, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.

[20] M. Marduel, K. Ouguerram, V. Serre, D. Bonnefont-Rousselot, A. Marques-Pinheiro, K.E. Berge, M. Devillers, G. Luc, J.M. Lecerf, L. Tosoloni, D. Erlrich, G.M. Pelosi, N. Vivona, G. Emmanuele, A.B. Cefalu, L. Pisciotta, V. Guido, D. Noto, B. Fiore, C.M. Barbagallo, A. Notarbartolo, S. Travali, S. Bertolini, M.R. Averna, Two Italian kindreds carrying the Arg154Cys mutation in codon 95/96 that is predicted to cause a premature stop codon, Ann. Clin. Biochem. 42 (2005) 264–268.