Case Report

MTP Gene Variants and Response to Lomitapide in Patients with Homozygous Familial Hypercholesterolemia

Genovefa D Kolovou¹, Vana Kolovou¹,², Anna Papadopoulou³ and Gerald F Watts⁴

¹Cardiology Department, Onassis Cardiac Surgery Center Athens, Greece
²Molecular Immunology Laboratory, Onassis Cardiac Surgery Center Athens, Greece
³DNAGENETIX, Medical Diagnostic Laboratory, 29 Andrea Papandreou str, Maroussi, Athens, Greece
⁴Lipid Disorders Clinic, Cardiovascular Medicine, Royal Perth Hospital School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia

Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disorder, which leads to premature cardiovascular diseases. Microsomal triglyceride transport protein (MTP) inhibitors, such as lomitapide, offer a new therapeutic approach for treating these patients. We evaluated the lipid lowering (LL) efficacy of lomitapide according to several gene variants in MTP. Four clinically and/or molecularly defined HoFH patients were treated with lomitapide in addition to conventional high intensity LL therapy and regular lipoprotein apheresis. Two patients responded to the therapy, with a significant reduction of LDL cholesterol (LDL-C) ≥50%, hyper-responders. Sequencing of all exonic and intronic flanking regions of the MTP gene in all patients revealed 36 different variants. The hyper-responders to lomitapide shared six common variants: rs17533489, rs79194015, rs745075, rs41275715, rs1491246, and rs17533517, which were not seen in hypo-responders (reduction in LDL-C <50%). We suggest that in HoFH variants in the MTP gene may impact on the therapeutic response to lomitapide, but this requires further investigation.

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Key words: Homozygous familial hypercholesterolemia, Microsomal transfer protein, Lomitapide, MTP gene

Introduction

Homozygous familial hypercholesterolemia (HoFH) is characterized by the following: extreme elevation of plasma low density lipoprotein cholesterol (LDL-C); accumulation of cholesterol in the corneas, eyelids, and extensor tendons; premature cardiovascular disease (CVD); and aortic valve disease¹,². Recent evidence suggests that the condition may be as frequent as 1 in 160,000–300,000 individuals³. Severe and resistant cases are treated with LDL apheresis and high intensity statin based lowering (LL) drug therapy⁴. Furthermore, new therapies such as the use of proprotein convertase subtilisin kexin type-9 (PSCK9) inhibitors, mipomersen and lomitapide [microsomal triglyceride transfer protein (MTP) inhibitor] may be valuable in these patients⁵, but responsiveness in LDL-C can be variable and genetically determined.

Aim

In a prospective case series, we investigated whether the LL efficacy of lomitapide in HoFH patients was dependent on MTP gene variants.

Methods

Four patients (N1, N2, N3, and N4) diagnosed clinically and genetically (N1 and N2) as HoFH, were treated with diet and maximum doses of available LL drug combination (rosuvastatin plus ezetimibe plus colesvelam) and regular LDL apheresis sessions (every
homozygous for c.1285G>A (p.Val429Met) resulting in <2% of LDLR function]. In patients N3 and N4, the DNA testing for FH was not carried out. The duration of lomitapide treatment was 14, 13, 7 and 6 months in patients N1, N2, N3, and N4, respectively. Demographic and clinical data of all patients are presented in Table 1. MTP gene analysis was performed in all patients. Briefly, genomic DNA was isolated from whole blood using the High Pure PCR Template Preparation Kit (Roche). Investigation of genetic changes in the 26 exons and flanking intronic regions of the MTP gene was performed using PCR and direct sequencing of PCR products. The PCR product was sequenced using the ABI3500 genetic analyzer. Sequence analysis was performed by the Seqscape module using ENSG00000144285/ENST00000303395 as reference DNA sequence.

Because no information is available for most of the MTP gene variants in the literature, the hypothetical approach was to define common gene variants that were associated with response to lomitapide.
Table 2. Interval mean values for plasma lipid and lipoprotein concentrations before and mean values (patients N2 and N4) and interval mean values (patients N1 and N3) after lomitapide treatment in the HoFH patients

| Patient | Before | After | Changes | % Changes |
|---------|--------|-------|---------|-----------|
| N1      |        |       |         |           |
| TC      | 527±72 | 329±49| -198    | -38       |
| TG      | 128±51 | 64±16 | -64     | -50       |
| HDL     | 30±6   | 32±5  | 2       | 7         |
| LDL     | 472±65 | 305±51| -167    | -35       |
| N2      |        |       |         |           |
| TC      | 388±23 | 177±15| -211    | -55       |
| TG      | 142±35 | 102±55| -40     | -28       |
| HDL     | 32±2   | 33±2  | 1       | 3         |
| LDL     | 330±25 | 114±15| -216    | -65       |
| N3      |        |       |         |           |
| TC      | 295±24 | 236±47| -59     | -20       |
| TG      | 105±38 | 69±19 | -36     | -34       |
| HDL     | 27±3   | 25±4  | -2      | -7        |
| LDL     | 220±27 | 196±46| -24     | -11       |
| N4      |        |       |         |           |
| TC      | 322±40 | 180±25| 142     | -45       |
| TG      | 114±13 | 70±22 | -34     | -39       |
| HDL     | 40±2   | 45±6  | 5       | 12        |
| LDL     | 259±38 | 118±30| -141    | -54       |

Before indicates the TC, TG, HDL and LDL values before adding lomitapide to regularly treatment with lipid lowering drugs and LDL apheresis sessions. In patients N2 and N4 “After” indicates mean values for blood lipids with lomitapide treatment and no LDL apheresis sessions and in patients N1 and N3 “After” indicates interval mean values after adding lomitapide and with LDL apheresis sessions. All data were expressed as mg/dl, mean±SD. The mean data were based on last 5 measurements before and Lomitapide administration. To convert cholesterol values (TC, HDL, and LDL) from mg/dl to mmol/l, multiply by 0.0259, and, for TGs, multiply by 0.0113. TC indicates total cholesterol, TG indicates triglycerides, LDL indicates low-density lipoprotein, HDL indicates high-density lipoprotein.

Clinical Criteria for the Diagnosis of HoFH

The diagnosis of HoFH in patients N3 and N4 were made on the basis of clinical criteria suggested by the Consensus Panel on Familial Hypercholesterolemia of the European Atherosclerosis Society

Results

Patients with the clinical criteria of HoFH (N3 and N4) had total cholesterol 1,000 and 800 mg/dl, respectively before any treatment of (Table 1), tendon xanthomata before 10 years of age, and both parents with definite HeFH diagnosed by Dutch Lipid Clinic Network criteria.

Both hyper-responders were treated with lomitapide 10 mg daily and the hypo-responders with 40 mg/daily (N1) and 30 mg/daily (N3). Patients N2 and N4 showed the highest reduction in plasma LDL-C concentration after the addition of lomitapide and were classified as hyper-responders (Table 2). Patients N1 and Patient N3 responded to lomitapide treatment with a lower reduction in LDL-C (Table 2) and required additionally LDL apheresis sessions to reach the treatment target and were classified as hypo-responders. As shown in Table 3, 36 different MTP gene variants were found in all patients. The hyper-responders (N2 and N4) shared six MTP gene variants (rs17533489, rs79194015, rs745075, rs41275715, rs1491246, and rs17533517). There was no sharing of
these variants in hypo-responders (N1 and N3). Five variants (rs991811, rs2306985, rs881981, rs2718684, and rs3816873) were common to 3 or to 4 patients (hyper-/hypo-responders), and thus, were not associated with LL therapeutic response to lomitapide. Patients N1 (hypo-responder) and N2 (hyper-responder) had seven common variants (rs17029213, rs17029215, rs34734558, rs79194015, rs2306984, rs34883891, and rs7667001), and thus, were not associated with the LL response. The variants exclusive to one hypo- or hyper-responder (Patient N1: rs2306984, rs2298747, rs3792681, rs991811, and rs1702921; Patient N2: rs17029189, rs113557405, rs112568939, rs113337987, and rs112407688; Patient N3: rs3833621, rs2306986, rs3792683, rs982424, and rs2255119; and Patient N4: rs61733139, rs114681504, rs1491244, and rs74542928) suggest the LL effect of lomitapide only in a particular patient, Table 4.

**Discussion**

MTP inhibitors offer a new therapeutic approach that has been FDA and EMA approved for HoFH patients; however, the reduction in LDL-C is variable. Careful attention to diet and safety monitoring (liver function and gastrointestinal complains) is necessary when using this agent. Raper et al. reported a HoFH patient treated for 5 years with lomitapide who achieved a marked reduction in LDL-C concentration, reaching the desirable target level of 70 mg/dl. Cuchel et al. reported overall reductions in LDL-C from baseline by 50% with the median dose of lomitapide of 40 mg/daily. Furthermore, of the 23 patients who completed the trial 16 achieved the therapeutic goals, with LDL-C of 100 mg/dl achieved by 8. In our study, we treated four HoFH (two with documented <2% of LDLR function) patients with lomitapide for a prolonged period of 14 months. Two patients had an extremely good response to lomitapide, with LDL-C reductions of 54% and 65% (hyper-responders), compared with two others with LDL-C reductions of <35% (hypo-responders). The reasons for the excellent response of the former two are unknown. All patients were given the similar diet, had desirable body mass index, and were treated with the same LL drugs and dose. That hyper-responders to lomitapide required lower doses of this drug than hypo-responders provides further internal consistency to our case definition and study hypothesis. One
regarding hypolipidemic drugs variants (e.g., CETP, apoE, LDL receptor) that regulate lipid metabolism. With lomitapide, the encoding \( MTP \) gene variant may improve the management of such difficult patients. Reliable inferences based on data from only four patients are questionable. However, the differences reported between hypo- and hyper-responders in our investigation were sufficiently clear to allow us to generate a good hypothesis that can be tested in future studies.

We suggest that the six SNPs \( rs17533489, rs79194015, rs745075, rs41275715, rs1491246, rs17533517 \) of the \( MTP \) gene recorded after sequencing of the whole \( MTP \) gene may influence the LL response to lomitapide because they were only found in hyper-responders. The \( MTP \) gene variants can potentially influence the LL effect of lomitapide or may be involved in a more complex network of gene–gene or gene–environment interaction, in which certain variants may influence the function of the \( MTP \) protein. Moreover, individually, the effect of the gene variants studied on the LL response to lomitapide is probably small, but collectively, it could have a significant stacking effect on the therapeutic response to this drug. Further studies are required to elucidate the role of these gene variants.

We suggest that the six SNPs \( rs17533489, rs79194015, rs745075, rs41275715, rs1491246, rs17533517 \) (C/T allele) of the \( MTP \) gene recorded after sequencing of the whole \( MTP \) gene may influence the LL response to lomitapide because they were only found in hyper-responders. The \( MTP \) gene variants can potentially influence the LL effect of lomitapide or may be involved in a more complex network of gene–gene or gene–environment interaction, in which certain variants may influence the function of the \( MTP \) protein. Moreover, individually, the effect of the gene variants studied on the LL response to lomitapide is probably small, but collectively, it could have a significant stacking effect on the therapeutic response to this drug. Further studies are required to elucidate the role of these gene variants.

The small number of the patients studied is one of the limitations of our work. However, this is owing to an extremely rare prevalence of HoFH and the involvement of only one center. According to historical prevalence data (one in a million), there are 900

| Table 4. MTP gene variants categorized by patient or groups of patients |
|-----------------------------|-----------------------------|
| Patients: N1, N2, N3, N4    |
| \( rs991811, rs2306985, rs881981 \) |
| Patients: N1, N2, N3        |
| \( rs2718684 \)              |
| Patients: N2, N3, N4        |
| \( rs3816873 \)              |
| Patients: N1, N2            |
| \( rs17029213, rs17029215, rs34734558, rs41275719, rs371030218, rs34883891, rs7667001 \) |
| Patients: N2, N4            |
| \( rs17533489, rs79194015, rs745075, rs41275715, rs1491246, rs17533517 \) |
| Patient N1                  |
| \( rs2306984, rs2298747, rs3792681, rs170291 \) |
| Patient N2                  |
| \( rs17029189, rs113557405, rs112568939, rs11337987, rs112407688 \) |
| Patient N3                  |
| \( rs3833621, rs2306986, rs3792683, rs982424, rs2255119 \) |
| Patient N4                  |
| \( rs61733139, rs114681504, rs1491244, rs74542928 \) |
individuals in the European region; however, according to extrapolation from recent Danish general population evaluated by Nordestgaard et al.13) (~1 in 160,000), there are 5,630 individuals in the European region. Furthermore, the diagnosis of HoFH is still underdiagnosed.

Conclusion

The absence of convincing explanation regarding the different response of patients under the same therapeutic protocol strengthens our hypothesis of a potential contribution of MTP variants in the LL effect of lomitapide. This case series is only a hypothesis, but the findings suggest that further investigation is warranted to establish whether MTP gene variants determine the therapeutic response of LDL-C to lomitapide in FH and that knowing the likely response based on gene score could enrich the decision making and physician and patient expectations in the future.

Conflicts of Interest

None.

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