Gene Therapy With Lipoprotein Lipase Variant S447X

To the Editor:

Ross et al recently reported a dazzling series of in vivo experiments showing reversal of abnormal biochemical phenotypes in Lpl”/” mice through adenoviral-mediated gene transfer of the so-called “gain-of-function” S447X prematurely truncated human variant of lipoprotein lipase (LPL or LIPID). Furthermore, all readouts in lipase-deficient mice treated with this human variant were at least as good as (and usually better than) those in mice treated with wild-type human LPL, providing some of the best evidence to date for a “gain-of-function” associated with this human variant, albeit in mice. These experiments offer hope for LPL-deficient patients, whose life quality and duration are compromised by their dyslipidemia. Indeed, somatic gene transfer experiments using adeno-associated virus (AAV) to deliver S447X (AAV1-LPLS447X) into the muscle of some patients with elevated plasma triglycerides (TGs) and low plasma LPL activity are underway.2,3

As this groundbreaking work proceeds, 2 points are worth remembering. First, the LPL S447X variant was initially discovered in patients with severe hypertriglyceridemia through genomic DNA screening experiments that were designed to detect loss-of-function mutations in LPL.4 One of these was my patient: a heterozygous woman of European ancestry 45 years of age with fasting chyloimicronemia and recurring pancreatitis, whose plasma TGs ranged between 25 and 80 mmol/L, despite good compliance with a low-fat diet and fibrate therapy. Her APOC2 was normal and her plasma postheparin LPL activity was ~50% of normal, similar to some patients in whom AAV-LPLS447X therapy is being contemplated.2,3 She had no other mutations in LPL and or in any other gene so far studied. In fact, S447X was originally implicated as being causative for her dyslipidemia, before its presence in many completely healthy individuals implied that it must be a normal variant.4 Another patient, a 30-year-old female, had plasma triglycerides ranging between 30 and 100 mmol/L and recurring pancreatitis, with depressed postheparin LPL activity and normal APOC2; she was a compound heterozygote for the LDL loss-of-function mutation G188E on one allele and S447X on the other allele. Thus, suggestions that somatic transfer of AAV-LPLS447X may help patients with LPL deficiency and perhaps those with other molecular forms of hypertriglyceridemia5 must be considered with caution. Certainly, germline presence of S447X did not protect some patients against severe hypertriglyceridemia.6 Patients with pure homozygous molecularly proven LPL deficiency (ie, analogues of the Lpl”/” mouse) rather than those with other forms of hypertriglyceridemia would be expected to benefit the most.

The second point is that without parallel-blinded somatic transfer using wild-type LPL, any benefit observed with somatic transfer of AAV-LPLS447X will not resolve the issue, which, for some of us, is still contentious of whether S447X really has a biologically or clinically meaningful “beneficial” or “gain-of-function” advantage over wild-type LPL in humans. Persistent attribution of a “gain-of-function” to this nonsense variant during human somatic gene transfer experiments in the absence of appropriate controls with wild-type LPL might be unintentionally misleading, especially if it is ever shown that similar clinical outcomes could have been achieved using the wild-type LPL gene.

Acknowledgments

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In Response:

We would like to thank Dr Hegele for his comments on our publication describing the preclinical results of gene therapy in a mouse model of lipoprotein lipase (LPL) deficiency.1 The potential for using LPL gene therapy to treat this disease has been investigated extensively,2–6 most recently using an adeno-associated viral vector (AAV) to express the LPL variant S447X in skeletal muscle of LPL-deficient mice.5 Since then, preclinical toxicity and biodistribution studies have led to regulatory approval for the clinical testing of this gene therapy vector in LPL-deficient patients.7

Dr Hegele raises 2 points. The first is that LPLS447X gene therapy may not be effective for all forms of hypertriglyceridemia. The second is related to the use of the S447X variant instead of the wild-type gene. We agree that LPL gene therapy may not overcome all causes of hypertriglyceridemia, and this is not our aim. We are hopeful that LPL gene therapy will reduce plasma triglycerides in patients with hypertriglyceridemia caused by reduced LPL enzyme activity. For this reason, the clinical trial of AAV1-LPLS447X requires that all patients must have proven genetic LPL deficiency confirmed by DNA sequence analysis of the LPL gene.7

Referring to the report from Hata et al, Dr Hegele points out that “germ line presence of S447X did not protect at least three patients with hypertriglyceridemia.” However, there are conditions for which the presence of the S447X variant would not be expected to overcome hypertriglyceridemia, such as ApoCII deficiency or inhibitory anti-LPL autoantibodies in some patients with autoimmune disease,8 and these patients would not be considered candidates for LPL gene therapy. Moreover, S447X carriers with additional LPL gene mutations would also be expected to exhibit hypertriglyceridemia. For example, Faustinella et al described patients with hypertriglyceridemia caused by homozygous Asp156Gly mutations in the LPL gene, who were also carriers of the S447X variant.9 Therefore, the beneficial effects of the S447X variant are not absolute, and other causes of hypertriglyceridemia should be taken into account.

Dr Hegele also questions the use of the S447X variant, and not wild-type LPL, as a therapeutic approach. The S447X variant was first discovered by Hata et al in an effort to identify mutations in hypertriglyceridemic patients. The S447X variant was present in 33% of normal controls (n=86), including 2 homozygous S447X carriers, and in only 9% of hypertriglyceridemic cases (P=0.037),9 and this provided the first evidence that the S447X variant may actually be a beneficial mutation. This finding has been replicated 27 times,10–36 demonstrating that LPLS447X is a beneficial variant asso-

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Plasma TGs, HDL Cholesterol, and Cardiovascular Disease Risk in Human LPL<sup>S447X</sup> Carriers

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Plasma TGs (Δ% vs WT)</th>
<th>P</th>
<th>Plasma HDL (Δ% vs WT)</th>
<th>P</th>
<th>Cardiovascular Disease Risk (odds ratio)</th>
<th>P</th>
<th>n (Carriers/noncarriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Hallman et al</td>
<td>↓ Reduced</td>
<td>&lt;0.05</td>
<td>Increased</td>
<td>&lt;0.05</td>
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<td></td>
<td>151/855</td>
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<td>2001</td>
<td>Ukkola et al</td>
<td>↓ 8.5%</td>
<td>0.03</td>
<td>↑ 8.6%</td>
<td>&lt;0.001</td>
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<td>2001</td>
<td>Shimo-N. et al</td>
<td>↓ 10%</td>
<td>NS</td>
<td>↑ 3%</td>
<td>NS</td>
<td>↓ CVD (0.68)</td>
<td>0.03</td>
<td>88/266</td>
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<td>2001</td>
<td>McGladdery et al</td>
<td>↓ 11.1%</td>
<td>NS</td>
<td>↑ 7.7%</td>
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<td></td>
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<td>2001</td>
<td>Clee et al</td>
<td>↓ 20.4%</td>
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<td>↓ CVD (0.61)</td>
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<td>2001</td>
<td>Chen et al</td>
<td>↓ 18.7%</td>
<td>&lt;0.01</td>
<td>↑ 2.8%</td>
<td>&lt;0.05</td>
<td>↓ CAD&lt;sup&gt;2&lt;/sup&gt; (0.49)</td>
<td>0.02</td>
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<td>2000</td>
<td>Garenc et al</td>
<td>↓ 21.8%</td>
<td>&lt;0.01</td>
<td>↑ 4.4%&lt;sup&gt;n&lt;/sup&gt;</td>
<td>NS</td>
<td></td>
<td></td>
<td>83/392</td>
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<td>2000</td>
<td>Sass et al</td>
<td>↓ 13.3%</td>
<td>&lt;0.01</td>
<td>↑ 14%&lt;sup&gt;n&lt;/sup&gt;</td>
<td>NS</td>
<td></td>
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<td>354/1229</td>
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<td>1999</td>
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<td>↓ 14%</td>
<td>0.02</td>
<td>↑ 5.3%</td>
<td>0.01</td>
<td>↓ CHD (0.43)</td>
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<td>↑ 3.7%</td>
<td>&lt;0.05</td>
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<td>112/396</td>
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<td>1998</td>
<td>Sass et al</td>
<td>↓ 23.6%</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39/111</td>
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<td>1998</td>
<td>Humbries et al</td>
<td>↓ 5.4%&lt;sup&gt;n&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>No difference</td>
<td></td>
<td>↓ MI&lt;sub&gt;y&lt;/sub&gt; (0.71)</td>
<td>&lt;0.05</td>
<td>303/1162</td>
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<td>1997</td>
<td>Kuivenhoven et al</td>
<td>nd</td>
<td></td>
<td>↑ Increased</td>
<td>&lt;0.001</td>
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<td>50/191</td>
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<td>1997</td>
<td>Groenemeijer et</td>
<td>↓ 8%</td>
<td>0.044</td>
<td>↑ 4.4%</td>
<td>0.013</td>
<td></td>
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<td>149/662</td>
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<td>1997</td>
<td>Salah et al</td>
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<td>0.01</td>
<td>↑ 4.4%</td>
<td>NS</td>
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<td>242/831</td>
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<td>1997</td>
<td>Knudsen et al</td>
<td>No difference</td>
<td></td>
<td>No difference</td>
<td></td>
<td>↓ HyperTG risk (0.32)</td>
<td>&lt;0.05</td>
<td>28/98</td>
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<td>1996</td>
<td>Galton et al</td>
<td>↓ Reduced</td>
<td>&lt;0.04</td>
<td>No difference</td>
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<td>↓ CVD (0.73)</td>
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<td>1995</td>
<td>Jema et al</td>
<td>↓ 10.1%</td>
<td>&lt;0.01</td>
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<td>↓ CHD (0.85)</td>
<td>NS</td>
<td>165/556</td>
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<td>1992</td>
<td>Stocks et al</td>
<td>↓ 4.5%</td>
<td>NS</td>
<td>↑ 4.8%</td>
<td>NS</td>
<td>↓ Lipidemia risk (0.52)</td>
<td>&lt;0.05</td>
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<tr>
<td>1990</td>
<td>Hata et al</td>
<td>nd</td>
<td></td>
<td></td>
<td></td>
<td>↓ HyperTG risk (0.27)</td>
<td>0.037</td>
<td>31/90</td>
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</table>

Studies showing no differences in plasma lipids or cardiovascular disease risk for S447X carriers

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Plasma TGs (Δ% vs WT)</th>
<th>P</th>
<th>Plasma HDL (Δ% vs WT)</th>
<th>P</th>
<th>Cardiovascular Disease Risk (odds ratio)</th>
<th>P</th>
<th>n (Carriers/noncarriers)</th>
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<tr>
<td>1996</td>
<td>Hegele et al&lt;sup&gt;17&lt;/sup&gt;</td>
<td>No difference</td>
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<td>No difference</td>
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<td>45/717</td>
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<tr>
<td>1994</td>
<td>Mattu et al&lt;sup&gt;18&lt;/sup&gt;</td>
<td>↓ 3%</td>
<td>NS</td>
<td>↑ 3%</td>
<td>NS</td>
<td></td>
<td></td>
<td>22/101</td>
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<td>1992</td>
<td>Peacock et al&lt;sup&gt;19&lt;/sup&gt;</td>
<td>No difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10/82</td>
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Unless noted otherwise, the studies group together S447X heterozygotes (S/X) and homozygotes (X/X), <sup>n</sup>males, <sup>n</sup>females, <sup>n</sup>heterozygous S447X carriers only, <sup>n</sup>homozygous S447X carriers only, <sup>n</sup>family history. CVD indicates cardiovascular disease; CAD, coronary artery disease; MI, myocardial infarction; nd, not determined.

The parallel testing of AAV-mediated delivery of LPL<sup>WT</sup> and LPL<sup>S447X</sup> in humans, although certainly ideal from a scientific standpoint, is unfortunately not a realistic option at this time because of costs of development and regulatory requirements. The naturally occurring LPL<sup>S447X</sup> was selected as a transgene because it shows beneficial improvements in lipid profiles in human S447X carriers, as demonstrated in the Table. Our article also demonstrates that expression of the S447X variant is a more potent triglyceride-lowering strategy than a similar one using wild-type LPL. Finally, a single administration of an AAV vector expressing LPL<sup>S447X</sup> shows long-term complete correction of the hypertriglyceridemia in LPL-deficient mice, and preclinical toxicity and biodistribution studies have led to regulatory approval for clinical testing in LPL-deficient patients. Together, these provide a strong rationale for choosing the S447X variant for gene therapy in LPL-deficient patients.

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