Gain-of-Function Mutations and Therapeutic Implications

Lipoprotein Lipase S447X to the Rescue

Daniel J. Rader

Somatic gene transfer has been extensively used to express genes of interest and probe the molecular physiology of lipoprotein metabolism and many other processes in animal models. Among its advantages over germ-line transgenic animals are the ability to rapidly express genes on selected genetic backgrounds without time-intensive backcrossing, the greater ability to control expression level through dosing of the gene transfer vector, and the greater ability to directly compare 2 genes, or 2 variants of the same gene, with regard to their effects in vivo. The latter advantage has not been fully exploited in the investigation of the functional consequences of naturally occurring human mutations.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* is a report by Ross and colleagues of the rescue of lipoprotein lipase (LPL)-deficient mice from lethality using neonatal intramuscular injection of an adenoviral vector to achieve somatic gene transfer and expression of a naturally occurring gain-of-function mutant of LPL. This report presents a variety of important issues for investigators interested in lipoprotein metabolism as well as those generally interested in somatic gene transfer and therapy. Unlike in humans, LPL deficiency in mice is lethal within the first 48 hours of birth. Whereas a previous study demonstrated that neonatal intraperitoneal administration of an adenoviral vector encoding wild-type LPL rescued 3% of the mice to adulthood, the current study achieved 95% rescue to adulthood. The major difference appears to be that Ross et al used a naturally-occurring variant of LPL, known as S447X, that has a premature nonsense codon 2 amino acids from the normal termination of the protein. The S447X variant is common, with ~20% of the population carrying at least 1 allele. Carriers have been shown to have reduced triglyceride (TG) and increased high-density lipoprotein cholesterol (HDL-C) levels and reduced risk of coronary heart disease (CHD). Indeed, there is some evidence that carriers have higher post-heparin plasma LPL activity, consistent with a gain of function. However, in vitro studies comparing S447X to wild-type LPL have not been definitive, and no in vivo studies had previously been reported.

Thus, the results reported by Ross et al, in which LPL-S447X was directly compared with wild-type LPL (LPL-WT) in vivo on the background of absent endogenous LPL activity, are particularly important. In contrast to the mice injected with the adenoviral vector encoding LPL-S447X, none of the mice injected with LPL-WT survived past 21 days despite similar levels of LPL protein expression. This experiment demonstrates that LPL-S447X is catalytically more effective and thus more capable of rescuing LPL-deficient mice, and as such constitutes the most convincing proof to date that S447X is in fact a gain-of-function mutation resulting in an enzyme with greater specific activity than wild-type LPL. Not surprisingly, the differences between the 2 forms of LPL in these studies in mice (functionally “homozygous” for either form of LPL) were more dramatic than between heterozygous human carriers and non-carriers of the S447X variant. It is curious that despite the relative frequency of this allele, there has been relatively little focus specifically on the phenotype of the homozygotes for S447X.

This study has implications for gene therapy for LPL deficiency, a recessive genetic disorder in which acute pancreatitis can be life-threatening and for which inadequate medical therapy exists. The ability to achieve adequate levels of LPL gene expression to reduce TG levels and prevent pancreatitis is one of the major potential barriers to successful gene therapy for this disease. The use of LPL-S447X rather than wild-type LPL would be expected to significantly increase lipolytic activity for a given amount of LPL protein expression and thus be that much more likely to achieve a therapeutic benefit. Adenovirus is not suitable for clinical gene therapy trials directed against chronic conditions attributable to transient expression; indeed, LPL-deficient mice rescued using the adenoviral vector approach described in the current report returned to being markedly hypertriglyceridemic as adults because of loss of expression of LPL-S447X after the neonatal adenoviral injection. However, adenoassociated virus (AAV) vectors have been used increasingly for somatic gene transfer in animal models and have the substantial advantage of providing longer-term relatively stable transgene expression compared with adenoviral vectors. AAV vectors have been demonstrated to be particularly effective in achieving transgene expression after intramuscular injection. Indeed, the same investigators reported last year that in adenovirally “rescued” adult LPL-deficient mice a single intramuscular injection of an AAV1-LPL-S447X vector markedly reduced TG levels and maintained near normal levels of TGs for >1 year. They also state in the current manuscript that they have achieved similar results (unpublished) in LPL-deficient cats using an intramuscular injection of an AAV1-based vector. Though these studies do not involve the same elegant direct comparison of LPL-S447X with wild-type LPL as the current study, it is reasonable to believe that the results in these AAV vector–based studies may not have been as positive if wild-type LPL had been used instead of LPL-S447X. In fact, this same group of investigators has announced the planned initiation of a clinical trial using intramuscular injection of AAV1-LPL-S447X in patients with LPL deficiency. If gene therapy using intramuscular injection of AAV1-LPL-S447X is successful, it will open the...
Door to other possible applications of LPL gene therapy including in patients with severe hypertriglyceridemia and relatively low LPL activity levels, and potentially even patients with low HDL-C levels and premature CHD. Furthermore, recent advances involving the discovery of novel AAV serotypes that are even more effective for somatic gene transfer ensure that this area will continue to evolve rapidly.

Gain-of-function mutations that are beneficial, such as LPL-S447X, are interesting in that they provide insights into the structure–function properties of a protein and can also have therapeutic implications as noted above. Proven examples of beneficial naturally-occurring germline gain of function mutations are relatively rare. One putative example, well-known in the field of lipoprotein metabolism and atherosclerosis, is that of apoA-I_S447X. A rare Arg173Cys point mutation in apoA-I. Heterozygosity for apoA-I_S447X is associated with very low levels of HDL-C but no apparent increased risk of CHD. Based on relatively little hard direct comparative data, apoA-I_S447X, is believed to be a gain-of-function mutation conferring increased antiatherogenic effects compared with wild-type apoA-I. ApoA-I_S447X has been studied extensively, including transgenic and somatic gene transfer studies of atherosclerosis in animals and even in a clinical trial of repeated intravenous infusion of an apoA-I_S447X/phospholipid complex in patients with CHD. However, there have been remarkably few studies comparing apoA-I_S447X directly to wild-type apoA-I in vivo and none with regard to atherosclerosis. AAV1-, AAV2-, and AAV5-based vectors have been used to express apoA-I_S447X at very low concentrations after intramuscular or intravenous injection, but no comparison with wild-type apoA-I was made. This is one example where a rigorous study comparable to that by Ross and colleagues using somatic gene transfer to compare the 2 forms of apoA-I head to head, ideally on an apoA-I–null background, in their effects on atherosclerosis would be welcome.

In summary, the use of somatic gene transfer provides the opportunity to directly compare 2 genetic forms of the same protein in vivo on the genetic background of choice to determine functional differences between them. The current study by Ross et al represents one of the clearest demonstrations to date of a naturally occurring gain-of-function variant that is physiologically more effective than the wild-type protein in vivo, an observation that may well turn out to have direct therapeutic implications.

References
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