Novel iatrogenic amyloidosis caused by peptide drug liraglutide: a clinical mimic of AL amyloidosis

Amyloidoses are a group of heterogeneous diseases caused by the extracellular deposition of insoluble proteins or peptides in a beta-pleated sheet pattern.[1-4] There are approximately 30 different proteins that are known to produce the disease in humans.[5] In addition, iatrogenic amyloidosis is a recognized complication of subcutaneous injection of peptide or protein drugs such as insulin and viral protein mimetics.[6,7] Iatrogenic amyloidosis is rare and, in the presence of a monoclonal gammopathy, can be presumed to be the more common disorder, systemic immunoglobulin-derived light chain (AL) amyloidosis. Accurate diagnosis is imperative because treatment of AL amyloidosis may require chemotherapy and/or stem cell transplantation while iatrogenic disease does not require treatment. Here we present a case of iatrogenic amyloidosis induced by subcutaneous injections of liraglutide for the management of diabetes.

An 81-year-old man with a history of melanoma, coronary artery disease, atrial fibrillation, hypertension, diabetes and peripheral neuropathy was found to have amyloid deposits in the subcutaneous tissue at the time of melanoma resection. He was clinically well and only noted longstanding, mild peripheral neuropathy. Cardiac disease and diabetes were effectively medically managed which included oral metformin and subcutaneous injections of peptide drug, liraglutide. The patient was referred for evaluation of systemic amyloidosis.

Serum studies identified an IgM lambda monoclonal gammopathy via immunofixation without an M-spike or elevated free lambda chains. Histological sections of the abdominal skin biopsy showed a pale pink amorphous material in the deep dermis and subcutis which was confirmed to be positive for extensive amyloid deposition by Congo red stain. Immunohistochemical studies for AL amyloidosis and for insulin-associated amyloidosis were negative. Given the presence of a monoclonal gammopathy without elevated light chains and a clinical concern for systemic AL amyloidosis based primarily on neuropathy, the specimen was submitted for mass spectrometry-based proteomic analysis.

Liquid chromatography-mass spectrometric (LC-MS) analysis of the microdissected amyloid deposits was performed on an UltiMate™ 3000 RSLCnano and Q Exactive Plus mass spectrometer (Thermo Fisher Scientific) as previously described.[8-10] Data was searched using Byonic within Proteome Discoverer (Thermo Fisher Scientific) against the UniProt human reference database

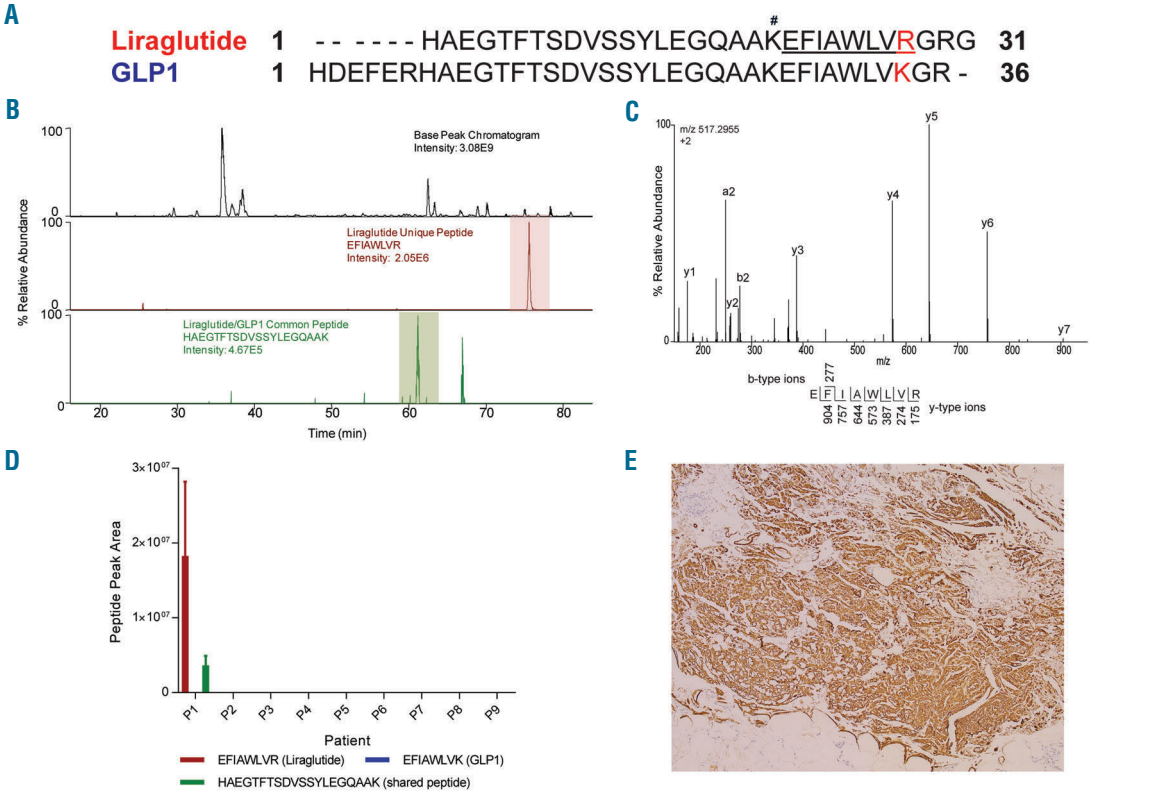

Figure 1. A liraglutide-specific peptide was detected via LC-MS analysis in the amyloid deposit biopsied from a patient with a history of liraglutide use and the amyloid deposit was positive for glucagon by immunohischemistry. (A) An alignment of the sequences of native GLP1 peptide and drug liraglutide shows a single amino acid change at position 28 (highlighted in red) in liraglutide which would generate a unique tryptic peptide (underlined). The # indicates the addition of γ-glutamic acid- palmitoyl to the side chain of the lysine in liraglutide. (B) Base peak chromatogram (top), extracted ion chromatogram of the +2 form of the unique liraglutide peptide (red) and the extracted ion chromatogram of the +3 form of the common liraglutide/GLP1 peptide (green). (C) An annotated HCD MS/MS spectrum of the +2 form of the unique liraglutide peptide showing complete fragment ion coverage and confident identification. (D) The unique liraglutide peptide and the peptide shared with GLP1 are detected in only the sample from the patient with a history of liraglutide (P1) use but not in patients with other types of amyloidosis (P2-P9; see Table 1 for details. (E) Immunohistochemistry using a polyclonal antibody against glucagon shows strong reactivity against subcutaneous amyloid deposits confirming mass spectrometry findings (Original magnification x40, image generated by Olympus BX53 microscope and DP27 camera using manufacturer's software, CellSense).

Table 1. The detection by LC-MS of amyloidosis protein markers and subtype markers in a liraglutide-induced amyloidosis patient sample and eight previously characterized amyloidosis samples.

Patient	P1			P2			P3			P4			P5			P6			P7			P8			P9		
Diagnosis	A Lir			AL Lambda			AL Lambda			AL Kappa			ALys			AKer			ACal			ATTR			AL Kappa		
Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
APOE	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
SAMP	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Subtype Protein Markers																											
Lambda LC				■	■	■	■	■	■																		
Kappa LC										■	■	■													■	■	■
Lysozyme													■	■	■												
Keratin 5																■	■	■									
Keratin 14																■	■	■									
Calcitonin																			■	■	■						
Transthyretin																						■	■	■			
Liraglutide	■	■	■																								

Green indicates the protein was detected in the amyloid deposits.

with the addition of immunoglobulin variant domains, liraglutide, glucagon-like 1 peptide (GLP1), and common contaminant sequences. Only high confidence peptide spectral matches (PSMs) with FDR of 1% or less and a Byonic score of 300 or higher are reported. Proteins with at least one unique peptide and five high-confidence PSMs are considered for clinical interpretation.

The results from eight patients with previously characterized and validated amyloidosis cases with no history of liraglutide use were compared to determine if liraglutide or GLP1 were detected in those amyloid deposits. Immunohistochemistry for glucagon was performed on formalin-fixed paraffin-embedded tissue using a polyclonal rabbit antibody (Ventana Medical Systems, Inc.) and standard techniques.

LC-MS analysis confirmed the diagnosis of amyloidosis with the detection of protein biomarkers of amyloid, apolipoprotein E and serum amyloid protein P with at least two unique peptides and five or more high confidence peptide spectral matches (PSMs) in the reported patient (P1 in Table 1) and eight previously characterized amyloidosis patient samples (P2 – P9 in Table 1). Known amyloid subtype protein markers were not detected in the patient samples (P1), but liraglutide was detected in all three replicate samples. Specifically, we detected a peptide that is shared by glucagon, GLP1 and liraglutide and a peptide that is unique to the sequence of liraglutide (Figure 1A-C). The peptide unique to GLP1 (not in liraglutide) was not detected in the patient sample indicating that the peptides were of exogenous origin (Figure 1D). None of the liraglutide or GLP1 peptides were detected in the eight previously characterized amyloidosis samples (Figure 1D). To confirm these findings, we developed an immunohistochemistry assay using a polyclonal antibody which can recognize epitopes shared by native glucagon and liraglutide. The amyloid deposits from the reported patient were strongly reactive with the anti-glucagon antibody and the other amyloid samples were negative (Figure 1E). These results indicated that the amyloid identified in the reported patient was the result of subcutaneously injected liraglutide and confidently ruled out other known causes of systemic amyloidosis, including AL amyloidosis.

This is the first reported case of liraglutide-induced amyloidosis and represents a new iatrogenic amyloid type. Glucagon encoded by the *GCG* gene is a preproprotein that is cleaved into four distinct mature peptides. One of these, GLP1 (also called glucagon) hormone, counteracts the glucose-lowering action of insulin by stimulating glycogenolysis and gluconeogenesis. Liraglutide is a GLP1 mimetic peptide drug, with approximately 94% amino acid homology to GLP1 (Figure 1A), which is administered subcutaneously for the management of diabetes. Like other peptide drugs such as insulin, repeated injections at the same site may lead to extensive localized amyloid deposition. This observation has two important implications. First, as has been described for insulin, once the amyloid plaque has developed, continued injection at the same site may lead to poor absorption and drug resistance.[11,12] More importantly, liraglutide-associated amyloidosis may be misdiagnosed and mismanaged as systemic AL amyloidosis.

The clinical syndromes of advanced diabetes and AL amyloidosis overlap, both causing renal disease and peripheral neuropathy. Monoclonal gammopathy is predicted to be present in approximately 5% of diabetics over the age of 70.[13] Therefore, in any patient at risk of having more than one amyloidogenic precursor protein, clinical and laboratory features are not sufficient to establish the nature of amyloid deposition. Abdominal subcutaneous fat aspirate biopsies are frequently used to establish the presence of systemic amyloidosis. Yet, in diabetic patients receiving abdominal subcutaneous injections of insulin or liraglutide, finding amyloid in the subcutaneous fat may lead to a misdiagnosis of systemic AL amyloidosis. In light of this and previous reports of other pharmaceutical-derived amyloidosis cases, a cautious approach is recommended when a diagnosis of systemic AL amyloidosis is based solely on a fat pad biopsy in a patient with a monoclonal gammopathy and diabetes. Amyloid typing by mass spectrometry-based proteomic approaches is recommended to rule out iatrogenic phar-

maceutical amyloidosis and/or confirm AL as the cause of amyloidosis.

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References

1. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. N Engl J Med. 2003;349(6):583-596.
2. Nuvolone M. PG, Merlini G. Amyloid diseases at the molecular level: general overview and focus on AL amyloidosis. In: Picken MD P, FASN M., Dogan, M.D., Ph.D. A., Herrera, M.D. G., ed. Amyloid and Related Disorders Current Clinical Pathology Humana Press; 2012.
3. Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. Annu Rev. Biochem. 2006;75(1):333-366.
4. Knowles TPJ, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. Nat. Rev. Mol. Cell Biol. 2014;15(6):384-396.
5. Sipe JD, Benson MD, Buxbaum JN, et al. Amyloid fibril proteins and amyloidosis: chemical identification and clinical classification International Society of Amyloidosis 2016 Nomenclature Guidelines. Amyloid. 2016;23(4):209-213.
6. D'Souza A, Theis JD, Vrana JA, Dogan A. Pharmaceutical amyloidosis associated with subcutaneous insulin and enfuvirtide administration. Amyloid. 2014;21(2):71-75.
7. Theis JD, Vrana JA, Dogan A. Drug-induced amyloidosis: a proteomic insight into 52 cases. Blood. 2013;122(21):1871-1871.
8. Klein CJ, Vrana JA, Theis JD, et al. Mass spectrometric–based proteomic analysis of amyloid neuropathy type in nerve tissue. Arch Neurol. 2011;68(2):195-199.
9. Rodriguez FJ, Gamez JD, Vrana JA, et al. Immunoglobulin derived depositions in the nervous system: novel mass spectrometry application for protein characterization in formalin-fixed tissues. Lab Invest. 2008;88(10):1024-1037.
10. Vrana JA, Gamez JD, Madden BJ, Theis JD, Bergen HR, Dogan A. Classification of amyloidosis by laser microdissection and mass spectrometry–based proteomic analysis in clinical biopsy specimens. Blood. 2009;114(24):4957-4959.
11. Albert SG, Obadiah J, Parseghian SA, Yadira Hurley M, Mooradian AD. Severe insulin resistance associated with subcutaneous amyloid deposition. Diabetes Res Clin Pract. 2007;75(3):374-376.
12. Buadi F, Dispenzieri A, Rizza R, Jacobson S, Rizza S, Dogan A. A case of insulin resistance secondary to insulin induced localized cutaneous amyloidosis. Blood. 2009;114(22):4908-4908.
13. Therneau TM, Kyle RA, Melton LJ, et al. Incidence of monoclonal gammopathy of undetermined significance and estimation of duration before first clinical recognition. Mayo Clin Proc. 2012; 87(11):1071-1079.