Genetic Analysis

DNA samples were analyzed by bi-directional fluorescent sequencing of all 17 exons of the VCP gene. Primer sequences were designed using the Primer-3 Plus program and are available upon request.

PCR reaction mix consisted of 50ng genomic DNA, 0.1 μmol of each primer, and Immolase (Bioline) in a reaction mix of 20μl. Amplification of exon 1 contained an additional 15% DMSO. Denaturation was performed at 95°C for 4 min and then followed by 32 cycles of 94°C for 1min, 60°C for 1min, and 72°C for 1min, with a final extension step of 72°C for 10 min.

PCR products were sequenced with the BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were size-separated on an Applied Biosystems 3500 xL Genetic Analyzer. Sequence data was analyzed with Mutation Surveyor version 4.0.8 software (Soft Genetics). Nomenclature was based on sequence accession number NM_007126.3 where the A of the initiation codon ATG is nucleotide 1.

Mutation analysis

In addition, three unrelated patients referred for VCP testing were found to have variants of unknown clinical significance: c.1360-14C>G, c.2214A>G and c.2316-46C>T. None of these three patients had a clinical phenotype strongly suggestive of IBMPFD. The c.2214A>G synonymous substitution is not predicted by bioinformatic analysis to be pathogenic. The intronic variants c.1360-14C>G and c.2316-46C>T are predicted by bioinformatic analysis to have a potential impact on splicing and we are aiming to undertake RNA studies to investigate these variants further once samples become available.