HDV full genome sequencing and genotype determination in a Berlin cohort

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AIM

HDV infection is widespread worldwide and between 10 and 20 million infections are reported. An infection with HDV occurs either through a superinfection or a coinfection and can lead to the development of liver cirrhosis and/or hepatocellular carcinomas. To date, eight different HDV genotypes have been described, which differ in the genome by up to 20%, while the individual subtypes have a homology of >90%. Germany has a low HDV prevalence due to a high HBV vaccination rate, with an estimated 3600 HDV positive people.
We adapted a sequencing system for the phylogenetic analysis of HDV in a Berlin cohort.

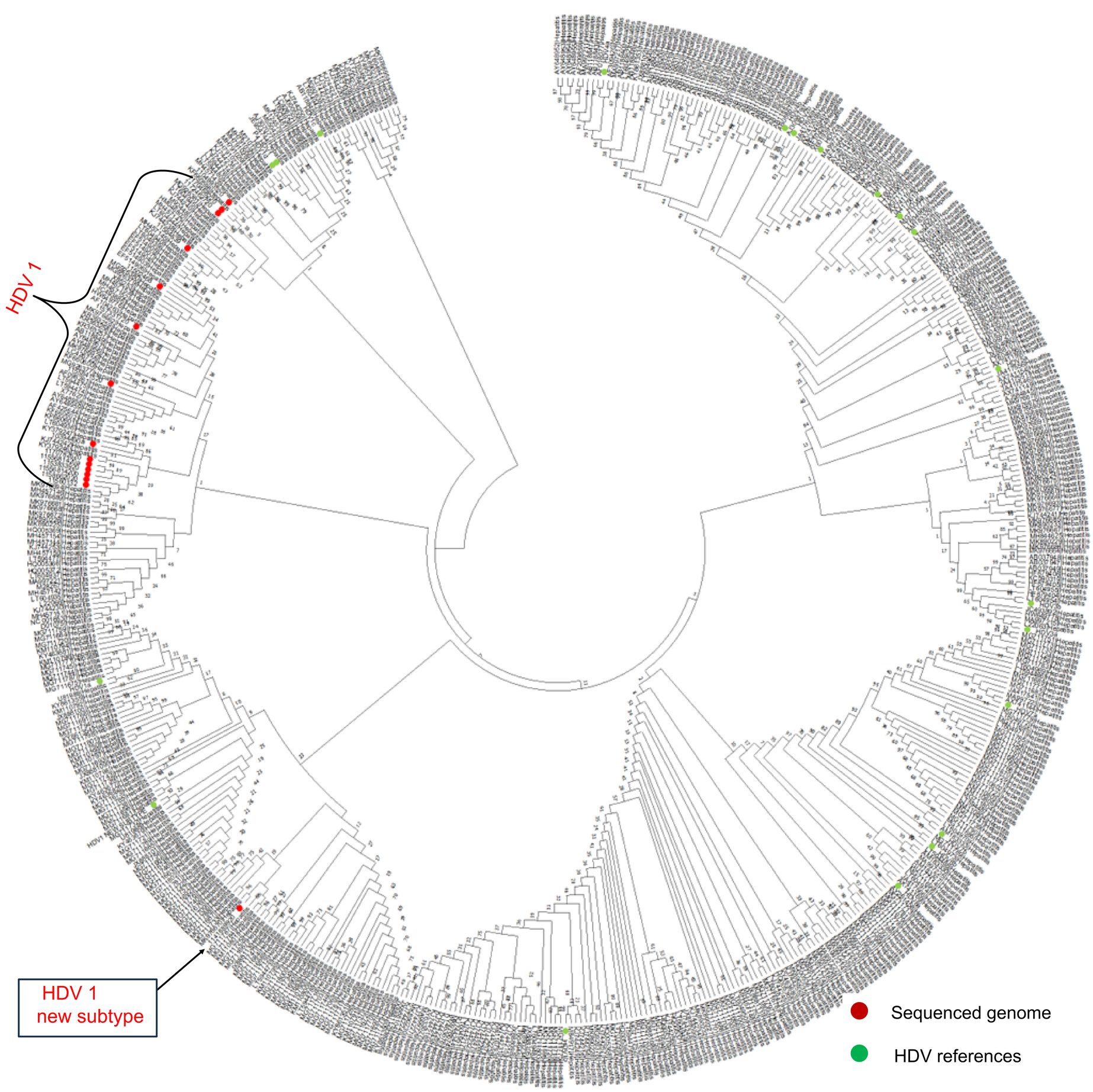
METHODS

Using whole genome sequencing with Nanopore technology (Oxford nanopore, UK), 18 HDV PCR-positive routine samples were analysed. RNA extraction was performed using 140µl plasma with QIAamp Mini Column (QIAGEN, Germany) and an elution volume of 80µl in AVE buffer. PCR was performed according to the protocol of Charre et al. 2023. After sequencing, the raw data was analysed using the Oxford Nanopore Technologies basecaller and the MARS (Multiple Alignment of Refined Sequences) tool. The phylogenetic analysis for genotype and subtype determination was carried out using the MEGA 11 programme.

RESULTS

Of the 18 samples analysed, a PCR product could be generated for sequencing in 17 cases, the failing sample had only 200 IU/mL HDV. Using the above described method, full length sequence of approximately 1,7 kilobases (kb) in size could be recovered. 15 sequences were completely analysable, all could be assigned to HDV genotype 1. This result reinforces the assumption that these people were probably infected in Europe. A cluster of 5 samples from 4 patients showed a small trans-mission cluster, while the other sequences were distri-buted throughout the available HDV genotype 1 full length se-quences.
As described by Charre C. et al. we could also find a sequence close to their newly discovered HDV 1 subtype which is more likely to be native to Africa and Asia. Based on this increasing amount of full length sequences the clustering of HDV subtypes is more complex than previously assumed. This is a relevant aspect for designing molecular diagnostic methods, quantifica-tion standards and most impor-tant therapeutic approaches.

Charre, C., Regue, H., Dény, P., Josset, L., Chemin, I., Zoulim, F., Scholtes, C., 2023.
Improved hepatitis delta virus genome characteriza-tion by single molecule full-length genome sequencing combined with VIRiONT pipeline. Journal of Medical Virology 95, e28634.
https://doi.org/10.1002/jmv.28634



Phylogenetic tree of the sequenced genotypes in conjunction with the HDV references and 500 other genotypes

CONCLUSIONS

The HDV genotype 1 is dominant in the analyzed cohort. With regard to migration from countries with higher HDV prevalence and other genotypes, the method used here offers the possibility to precisely characterize patient samples and to detect specific changes in the HDV genome in case of possible treatments of HDV with e.g. Bulevirtide. In addition, the method used makes it possible to clarify the countries of origin of new HDV variants in Berlin.

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