

Chapter 7

New Human Astroviruses

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Abstract The first human astrovirus was discovered in 1975 by Madeley and Cosgrove through visualization of viral particles in stool using electron microscopy. Over the course of the next ~20 years, an additional seven serotypes of human astroviruses were discovered. In the last decade, new technological advances in microarrays and sequencing strategies enabled more sophisticated methods for detecting viruses in clinical and environmental specimens. These methods led to the discovery of many novel viruses from a variety of virus families. They also brought about a dramatic realization that more astroviruses can be found in humans than previously recognized. In fact, the number of astroviruses associated with humans has nearly doubled within the last few years. Furthermore, the discovery of novel astroviruses in human specimens revealed that there is more diversity amongst them than was assumed based on the high level of similarity between human astroviruses 1 and 8. This chapter will describe the discovery and early characterization of the five novel astroviruses initially identified in human stool samples in 2008 and 2009.

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Introduction

The first human astrovirus was discovered in 1975 by Madeley and Cosgrove [34]. Over the course of the next ~20 years, an additional seven serotypes of human astroviruses were discovered [5, 21, 29, 31, 32]. However, new technological advances in virus discovery and detection methods have brought about a dramatic realization that more astroviruses can be found in humans than previously recognized and that there is more diversity amongst them than was assumed based on comparisons of human astroviruses 1–8. This chapter will describe the discovery and early characterization of the five novel astroviruses initially identified in human stool samples in 2008 and 2009.

Novel Virus Discovery Methods

Based on the tenets of Koch's postulates [17], the ability to grow a virus was long held as an essential step in its identification. However, enteric viruses have been notoriously difficult to grow in culture and in fact this has been the biggest ongoing challenge for the last 40 years in the human norovirus field [11]. The successful cultivation of some enteric viruses has depended on the inclusion of additives to the cultures (trypsin, pancreatin, bile, etc.) and/or serial blind passages in culture [3, 6, 49]. Even so, the ability to cultivate a virus does not inherently lead to its identification and characterization. In fact, visualization of viruses by electron microscopy (EM) was long used for the initial characterization of viruses that had been grown in culture or those present in stool samples from patients with diarrhea. In the 1970s, these methods were used to identify viral causes of diarrhea. The four families of viruses most commonly associated with diarrhea (*Rotaviridae*, *Caliciviridae*, *Astroviridae*, and *Adenovirus*) were all identified using EM [10, 18, 26, 34]. The identification of human astrovirus 1 was based on the observation of 28 nm virus particles present in stool from a child with gastroenteritis [34]. While these methods led to major advances in our understanding of the causes of diarrhea, they have limited our ability to detect a large number of viruses due to the fact that many viruses do not grow well, or at all, in cell culture and due to the relatively low sensitivity of EM studies. It is often difficult to classify a virus based on EM observations because many viral families exhibit similar morphologies under the microscope and also only 10% of astrovirus particles exhibit the classical star-like morphology [37]. Furthermore, the use of serological assays to determine the identity of an unknown virus is limited by the need for the unknown virus to cross-react with specific reagents for an already recognized virus. The use of serological assays is also hampered by the requirement for *a priori* knowledge of the possible identity of the unknown virus since there are currently no pan-viral serological assays available.

PCR-Based Strategies for Novel Virus Detection

The age of molecular biology has had an impact on virology just as it has on many other fields. In particular, the invention of the polymerase chain reaction (PCR) in the 1980s made it easy to detect viral genomes. If the appropriate sequences are available, it is quite easy to design primers to detect a given virus of interest. Analogous to the use of primers that target 16S ribosomal genes of bacteria for identification and classification of bacterial species, primers can often be designed to highly conserved regions of the genomes of viruses from a particular family or lower taxa. Such PCR assays can be used to identify novel viruses which happen to be closely related to existing viruses. Various computational strategies have been described for the specific design of consensus PCR primers to use for novel virus identification [24, 45]. The breadth of PCR assays is limited to interrogating a single taxon, which can be a limitation when there is no information to focus the efforts towards a particular group of viruses. However, there are numerous examples of viruses discovered in this fashion, with two notable instances being the identification of sin nombre virus [40] and human coronavirus HKU1 [53]. As will be discussed later in this chapter, PCR assays that were designed after the discovery of novel astrovirus MLB1 were used to identify additional novel astroviruses.

DNA Sequencing Methods for Novel Virus Detection

Dideoxy sequencing or Sanger sequencing, first described in 1977 [46], was the dominant DNA sequencing technology used for nearly 30 years. The typical sequence read length for Sanger sequencing can reach ~750–800 bp. Sanger sequencing is carried out individually for each sequence, so the number of sequences that can be generated for a single sample is simply a function of cost and time. This technology was the cornerstone of all significant sequencing projects up until ~2005, including the sequencing of the human genome. Starting in 2005, multiple new sequencing technology platforms (“NextGen”) emerged that have now far surpassed conventional Sanger sequencing in terms of increased total sequence production capacity and decreased cost. With NextGen platforms, sequences can be modified to encode specific strings of bases that serve as barcodes for individual samples, such that many samples can be combined into a single run on the NextGen machines. The NextGen platform that has been most widely used for virus detection and sequencing is 454 pyrosequencing [44]. At the time of the studies described in this chapter, one 454 sequencing run produced ~400,000 sequence reads of an average read length of ~250–400 bp [35]. Both Sanger sequencing and 454 pyrosequencing have been used in the discovery and sequencing of novel astroviruses.

Novel Astroviruses Found in Human Biological Samples

Astrovirus MLB1

Human rotaviruses and noroviruses are the leading causes of viral diarrhea [9, 42, 52] while human astroviruses have been associated with up to ~10% of sporadic cases of viral diarrhea in children [19, 51] and with 0.5–15% of outbreaks [2, 33, 48]. However, the cause of approximately 12–41% of the cases of sporadic diarrhea and gastroenteritis outbreaks remains undetermined despite extensive testing [25, 33, 48]. One hypothesis for the cause of such a diagnostic gap is that there are unidentified pathogens contributing to the burden of disease. This was the guiding principle of the initial study that led to the identification of Astrovirus MLB1 (AstV-MLB1) [12]. Stool samples from children with cases of acute, sporadic diarrhea were subjected to “micro-mass sequencing” meaning that only limited sequencing was done (384 sequences) per sample in contrast to the typically tens of thousands of sequences generated per sample using NextGen sequencing platforms that were used in studies described later in this chapter. One of the most interesting findings of the study was the detection of sequences, within one sample collected in 1999 from a 35-month-old child, that were most closely related to human astroviruses. These sequences were highly divergent from canonical human astroviruses 1–8, suggesting that a novel virus might be present. There were seven unique sequence reads derived from two loci that displayed 42–61% amino acid identity to human astroviruses. The sequence reads overlapped such that they could be assembled into two contiguous stretches of sequence referred to as contigs. RT-PCR was used to confirm the accuracy of the assembled contigs and subsequent sequencing of the amplicons confirmed the presence of the contigs in the original RNA extract. One contig was ~800 bp long and mapped to ORF1a, the serine protease, of the astrovirus genome and the other one was ~500 bp long and mapped to ORF1b, the RNA polymerase. Phylogenetic analysis of the two contigs yielded trees that demonstrated that these sequences in fact represented a novel astrovirus (Fig. 7.1).

This novel astrovirus was named Astrovirus MLB1 (AstV-MLB1) because it was identified in a stool sample from a child in Melbourne, Australia. In the follow-up study, the complete genome of AstV-MLB1 was sequenced to an average of >3X coverage [GenBank: FJ222451] and the virus was further characterized [14]. The AstV-MLB1 genome is organized like that of other astroviruses in that it is predicted to encode three open reading frames (ORF1a, ORF1b, and ORF2) and contain both 5' and 3' nontranslated regions (NTR), as well as a poly-A tail. AstV-MLB1 has a shorter genome than other astroviruses with only 6,171 bp (excluding the poly-A tail) compared to the range for the other astroviruses of ~6,400–7,300 bp [37] (Table 7.1). This is in large due to two deletions, totaling 57 amino acids, located within a highly conserved 144 amino acid motif near the carboxyl terminus of human astroviruses 1–8. The motif has been mapped as an immunoreactive epitope in human astroviruses [36] and is located in the nonstructural protein p38 [28], a protein reported to be involved in replication [20] and

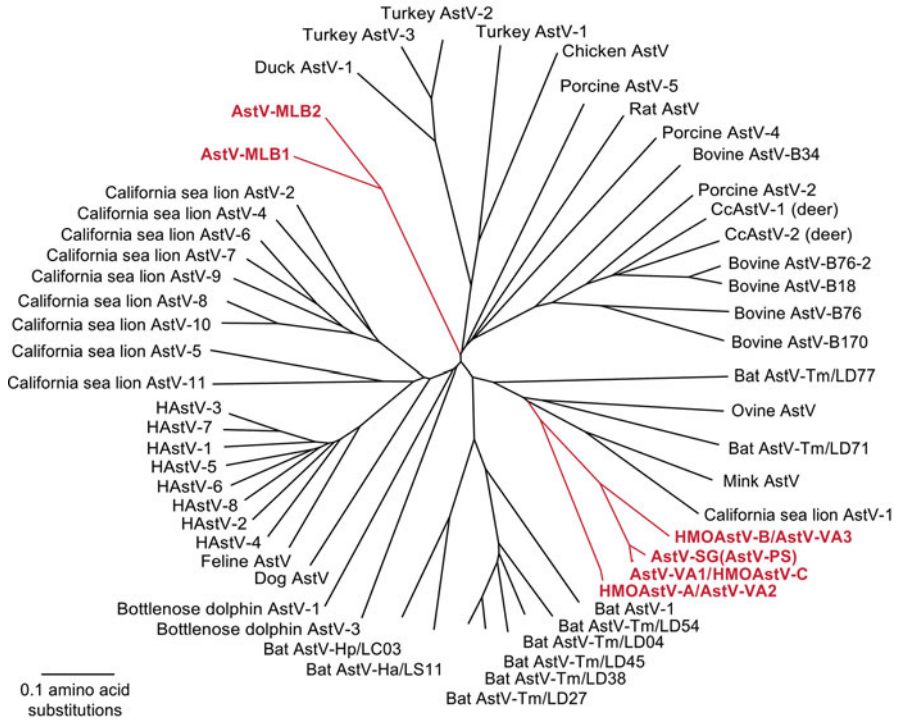


Fig. 7.1 Phylogenetic relationship of animal and human astroviruses based on capsid sequences. The neighbor-joining tree was generated using ClustalX2 [30] with 1,000 bootstrap replicates. *HAstV* human astrovirus. Novel astroviruses recently discovered in humans are shown in red

particle release [38]. The effect this deletion has on the replication of AstV-MLB1 has yet to be determined. Another difference between AstV-MLB1 and the canonical human astroviruses arises in the 3 C-like protease motif in the putative substrate binding residues. The canonical human astroviruses share the conserved residues RTQ whereas AstV-MLB1 is identical to ovine astrovirus with the residues ATR. Again, the meaning of this difference is yet to be explored.

AstV-MLB1 further differs from the canonical human astroviruses in some of its noncoding features. The 5' nontranslated region (NTR) is only 14 nucleotides long which is more comparable to the 5' NTRs of avian astroviruses (~10–20 nt) than to the 5' NTRs of human astroviruses 1–8 (80–85 nt long, 37) (Table 7.1). Furthermore, human astroviruses 1–8 contain a 120 nt region at the junction between ORF1b and ORF2 that is highly conserved with a 52 nt core region that is 99–100% identical among the human astrovirus serotypes [50]. It is hypothesized that this sequence serves as a regulatory element of the sub-genomic RNA that encodes the capsid. AstV-MLB1 is only 61.5% identical to other human astroviruses in the 52 nt core region. This is similar to the level of identity (44–59.6%) shared between the known animal astroviruses and the human astroviruses. Lastly, there is a conserved RNA secondary

Table 7.1 Genome Information of fully sequenced astroviruses

Virus	Alternate names	Genome (nt)	5' NTR (nt)	ORF 1a (nt)	ORF 1b (nt)	ORF 2 (nt)	3' NRT (nt)	GenBank accession #
California sea lion AstV-9	None	6,627	32	2,667	1,608	2,517	47	JN420356
Chicken AstV-1	Avian nephritis virus	6,927	15	3,017	1,533	2,052	305	NC_003790
Duck AstV-1	Duck hepatitis virus 2	7,722	22	3,723	1,551	2,196	217	F1919225
Turkey AstV-1	None	7,003	11	3,300	1,539	2,016	130	Y15936
Turkey AstV-2	None	7,325	21	3,378	1,584	2,175	196	NC_005790
Bovine AstV-B18	None	6,300	47	2,454	1,509	2,319	75	HQ916313
Bovine AstV-B76	None	6,253	44	2,448	1,509	2,280	76	HQ916316
Bovine AstV-B76-2	None	6,301	47	2,454	1,509	2,319	76	HQ916317
Bovine AstV-B170	None	6,317	23	2,448	1,467	2,358	83	HQ916314
Mink AstV	None	6,610	26	2,648	1,620	2,328	108	NC_004579
Ovine AstV	None	6,440	45	2,580	1,572	2,289	59	NC_002469
Porcine AstV-2	None	6,318	39	2,475	1,515	2,250	99	JF713710
Porcine AstV-4	None	6,639	74	2,553	1,515	2,478	97	JF13713
Porcine AstV-5	None	6,478	79	2,620	1,518	2,208	117	NC_001943
Human AstV-1	None	6,813	85	2,763	1,560	2,361	80	L13745
Human AstV-2	None	6,828	82	2,763	1,560	2,392	82	L13745
Human AstV-4	None	6,723	84	2,763	1,548	2,316	81	DQ070852
Human AstV-5	None	6,762	83	2,763	1,548	2,352	86	DQ028633
Human AstV-6	None	6,757	82	2,766	1,548	2,337	93	GQ495608
Human AstV-8	None	6,759	83	2,766	1,557	2,349	85	AF260508
AstV-MLB1	None	6,171	14	2,364	1,536	2,271	58	F1222451
AstV-MLB2	None	6,119	14	2,364	1,536	2,238	39	JF742759
AstV-VA1	HMOAstV-C, HastV-PS	6,586	38	2,661	1,575	2,277	98	F1973620
HastV-PS (HastV-SG in GenBank)	AstV-VA1, HMOAstV-C	6,584	39	2,658	1,575	2,277	98	GQ891990
HMOAstV-A	AstV-VA2	6,534	42	2,625	1,576	2,196	117	NC_013443
HMOAstV-B	AstV-VA3	No data	No data	No data	No data	2,267	No data	GQ415661

Genome information was obtained and/or deduced for all astroviruses in which full-length sequences were available at GenBank as of February 2012

structure, referred to as the stem-loop II-like motif (s2m), that is in the nontranslated region (NTR) at the 3' end of the genome in all previously described astroviruses except turkey astrovirus 2 [39]. It is thought to interact with viral and cellular proteins needed for RNA replication and to be quite important because (1) it is also found in some other RNA viruses and (2) compensatory mutations that restore base pairing are often seen when there are mutations in the conserved motif [39]. AstV-MLB1 not only lacks this conserved nucleotide motif but it also has the shortest 3'NTR reported to date for an astrovirus. It is not clear the importance of any of these differences in the coding or noncoding regions of the AstV-MLB1 genome compared to other astroviruses, but these may be interesting avenues of research in the future.

Astrovirus MLB2

The primer pair that has predominantly been used to identify astroviruses in stool samples, Mon269 and Mon270 [41], does not amplify AstV-MLB1. Since it was hypothesized that AstV-MLB1 might represent a whole new group of astroviruses, new primers were designed by Finkbeiner et al. based on an alignment of human astroviruses 1–8 and AstV-MLB1 in order to design new conserved primers that would detect all of these viruses. There are actually very few regions of conservation between AstV-MLB1 and the canonical human astroviruses in which primers can be designed and this is evidenced by the fact that two groups independently designed nearly identical primers [15, 54]. The primers were designed to ORF1b, the polymerase, and yield a ~400 bp amplicon.

These “pan-astroviral” primers were used to screen two cohorts of children with diarrhea: one in Vellore, India, and the other in St. Louis, MO, USA [13, 23]. The Vellore cohort comprised diarrhea samples collected in 2005 and 2006 from regular community sampling of a longitudinal birth cohort and from children hospitalized for acute gastroenteritis. The St. Louis cohort comprised samples sent for bacterial culture to the clinical microbiology laboratory at the St. Louis Children’s Hospital between June 2008 and March 2009. The initial intent of the study was to investigate the prevalence of AstV-MLB1, yet surprisingly this study also resulted in the discovery of a number of other novel astroviruses including one that was most similar to AstV-MLB1. It has provisionally been named AstV-MLB2 and shares ~80% amino acid identity to AstV-MLB1. It was detected in 2 out of 416 stools from Vellore and 3 out of 466 stools from St. Louis. AstV-MLB2 was later sequenced and determined to have a genome length of 6,119 nt [23] (Table 7.1).

Astrovirus VAI/Astrovirus HMO-C/HAstV-PS

Five fecal specimens were collected from a 1998 gastroenteritis outbreak at a child care center in Virginia, USA. There were 26 cases of illness in teachers and

children reported over a 2- to 3-week period. The samples tested negative for enteric parasites, bacteria, and viruses so they were subjected to high-throughput pyrosequencing and Sanger sequencing by two different groups that reported their findings together [16]. Unique sequences were detected in two of the samples by pyrosequencing: 313 sequences in one sample and 1,017 sequences in the other. Astrovirus-like sequences were assembled together into contigs of 6,376 nucleotides (nt) and 6,026 nt from the two samples. These contigs had limited sequence similarity (37–71% aa identity) to proteins from the mink and ovine astroviruses. When the two contigs were assembled together they generated a contig of 6,581 nt in length. The Sanger sequencing that was done simultaneously also resulted in the detection of unique astrovirus-like sequences in the same two samples: 3 in one sample and 69 in the other. Further RT-PCR amplifications and 5' RACE (rapid amplification of cDNA ends) reactions were used to determine that the full length of the virus is 6,586 nt, excluding the poly-A tail (Table 7.1). This virus has been provisionally named Astrovirus VA1 (AstV-VA1) because it came from an outbreak in Virginia.

AstV-VA1 has three predicted open reading frames and nontranslated regions (NTRs) at both the 5' and 3' ends of the genome consistent with the organization of other astroviruses. The lengths of the open reading frames and nontranslated regions are similar to those of mink and ovine astroviruses (Table 7.1). Like most other astroviruses (except TAstV-2 and AstV-MLB1), AstV-VA1 contains the stem-loop II-like motif that is putatively involved in replication. Phylogenetic analysis of the genome confirmed that while this virus is highly divergent from other astroviruses, at the time it was discovered it was most closely related to mink and ovine astroviruses with 61–62% amino acid similarity to them in ORF1b but only 39–42% for the other open reading frames (Fig. 7.1).

In addition to the two groups that both identified AstV-VA1 in association with the Virginia gastroenteritis outbreak, around the same time another group reported the discovery of novel astroviruses in diarrheal stools from Nepal and from stools of kids with nonpoliovirus acute flaccid paralysis in Nigeria and Pakistan [27]. They identified these viruses using similar primers as described above for the discovery of AstV-MLB2. One of the viruses they reported is AstV-VA1. They provisionally gave it the name HMO-C (HMOAstV-C), which stands for *human*, *mink*, and *ovine*-like astrovirus. HMOAstV-C was detected in 3 samples from Nepal out of a total of 328 samples between all of the locations.

Another virus that closely related to AstV-VA1/HMOAstV-C was identified in a 15-year-old patient with X-linked agammaglobulinemia and encephalitis and is described in greater detail later in this chapter [43]. The phylogenetic distance between this virus and AstV-VA1/HMOAstV-C is similar to the distances between human astroviruses 1 and 8, but much less than the distances between any of the other novel astroviruses or between the novel astroviruses and their closest relatives. This raises the question of how to name new viruses and how to define species, which is discussed at the end of this chapter.

Astrovirus HMO-A/Astrovirus VA2

Astrovirus HMO-A (HMOAstV-A), like AstV-VA1/HMOAstV-C, was discovered through PCR screening of a collection of stools from Nepal, Nigeria, and Pakistan [27]. Although seemingly most closely related to AstV-VA1/HMOAstV-C, the phylogenetic distance seems great enough for HMOAstV-A to be considered a different species (Fig. 7.1). It was found in two Nigerian children and one Pakistani child. Pyrosequencing, RT-PCR, and RACE reactions were used to determine the full length genome of HMOAstV-A. Excluding the poly-A tail, it is 6,534 nt long and shares the same organization as other astroviruses (Table 7.1). It contains other common genomic features of astroviruses including a ribosomal frameshift signal between ORF1a and ORF1b, a promoter sequence for putatively initiating ORF2 subgenomic RNA synthesis, a trypsin-like serine protease domain in ORF1a, a proteolytic cleavage site in ORF2, a viral genome-linked protein domain in ORF1a, and the s2m motif in the 3' NTR.

Just as with AstV-VA1/HMOAstV-C, there were concurrent discoveries of HMOAstV-A. It was also discovered in the same study in which AstV-MLB2 was discovered, involving PCR screening of stools from Vellore, India and St. Louis, MO, USA [13]. It was provisionally named AstV-VA2 in this study and was detected in two Indian stool samples and one American stool sample.

Astrovirus HMO-B/Astrovirus VA3

Astrovirus HMO-B (HMOAstV-B) was the third novel virus to be discovered by PCR in stools from Nepal, Nigeria, and Pakistan [27]. It was found in one sample from Nigeria and one from Pakistan. It is most closely related to the other VA/HMO astroviruses which are all more closely related to mink, ovine, and some of the newly discovered astroviruses in sea lions and bats than to human astroviruses 1–8 or AstV-MLB1 and AstV-MLB2 (Fig. 7.1). The genome was partially sequenced revealing that it has characteristics similar to AstV-VA1/HMOAstV-C and AstV-VA3/HMOAstV-A.

This was the third astrovirus that was discovered both by Finkbeiner et al. and Kapoor et al. in their respective PCR studies [13, 27]. Finkbeiner et al. provisionally named this virus Astrovirus VA3. In their study, it was detected in just one Indian stool making this the least prevalent of the novel VA/HMO astroviruses in both studies.

Epidemiology and Seroprevalence Studies

As described above, these new astroviruses have been found in multiple stool samples collected around the world. Specifically, AstV-MLB1 has been detected in the United States, Mexico, Hong Kong, Egypt, Nigeria, and India [1, 4, 8, 13, 27].

AstV-MLB2 has been detected in diarrhea stools in the United States and India [13]. AstV-VA1/HMOAstV-C has been found in stools from the United States, Nepal, and the Netherlands [16, 27, 43, 47]. AstV-VA2/HMOAstV-A has also been detected in stools from the United States, Egypt, India, and Nigeria [1, 13, 27]. Lastly, AstV-VA3/HMOAstV-B has been detected in stools from India and Pakistan [13, 27]. However, the role of these new viruses in human health and disease is still unknown. While the classic human astroviruses and most of the animal astroviruses are diarrheagenic, it is unknown if the novel human astroviruses are also agents of diarrhea. Ideally, proof that these viruses are a cause of diarrhea would come from human volunteer studies in which subjects would ingest fecal filtrates. However, these types of studies are no longer feasible for novel viruses of unknown pathogenicity. Therefore, proof of disease causality has to now come from indirect studies such as epidemiologic studies.

There has been one case-control study of AstV-MLB1 [22]. This study examined 400 acute diarrhea samples collected from children less than 3 years old in Vellore, India. These diarrhea samples were negative for rotavirus by enzyme immunoassay and PCR, for norovirus by PCR, for bacterial pathogens (*Vibrio cholerae*, enteropathogenic *Escherichia coli*, *Salmonella*, *Shigella*, *Aeromonas*, and *Plesiomonas*) by culture, biochemical reactions and serogrouping where appropriate and for parasites by routine saline and iodine preparations and modified acid fast stain. Control samples consisted of asymptomatic stool samples obtained from the same child at least 6 weeks prior to the collection of the diarrhea stool. These case and control stools were examined by astrovirus consensus primers which target the RNA polymerase [15]. Samples which were positive by the consensus primers were then screened by RT-PCR using primers specific for AstV-MLB1 and ones to detect the classic human astroviruses. In this study, 4 of the cases and 14 of the controls were positive for AstV-MLB1. Therefore, AstV-MLB1 was less likely to be present in the diarrheal samples than in the asymptomatic samples (OR 0.28, 95% CI 0.09–0.89, $p=0.033$).

Astrovirus VA1/HMO-C was found in the stool of one patient with diarrhea and new onset celiac disease [47]. Stools from four other patients with new onset celiac disease were tested for Ast-VA1 and were negative. Whether AstV-VA1 has a role in the development of celiac disease cannot be determined from this small study, but raises an interesting hypothesis that should be further studied.

To further understand if AstV-VA1/HMOAstV-C is truly a human pathogen or if it is a passenger from ingested food, Burbelo et al. conducted a seroprevalence study [7]. This study used a luciferase immunoprecipitation system to look at human IgG response to a C-terminal capsid fragment of AstV-VA1/HMOAstV-C. This study examined 106 healthy adult blood donors and found 65% to be seropositive for HMOAstV-C/AstV-VA1. Additionally, 103 age-stratified pediatric serum samples were examined. Seropositivity in the 6–12 month, 1–2 year, 2–5 year, and 5–10 year olds was 20%, 23%, 32%, and 36%, respectively. Additionally, it is likely there is transfer of maternal antibodies as 50% (11/22) of the 0–6 month old children showed anti-HMOAstV-C antibody responses. The findings of this study demonstrate that HMOAstV-C/AstV-VA1 is a prevalent infectious agent.

Interestingly, two of the novel astroviruses have been found outside of the enteric system. AstV-MLB2 was found in the plasma of a febrile child by mass sequencing, PCR, and quantitative PCR [23]. This 20-month old had a 3-day history of petechial rash, 1 day of fever, cough, and nasal congestion. The child did not have any vomiting or diarrhea. His evaluation included a normal leukocyte count and a negative blood culture. A nasopharyngeal swab was also negative for respiratory syncytial virus, influenza types A and B, parainfluenza, and adenovirus by fluorescent antibody testing, and culture results were negative for respiratory viruses. Chest X-ray results were interpreted as mild peribronchial thickening, which may represent a viral process. Additionally, plasma or blood of this patient was screened by PCR for the following viruses: adenovirus, enteroviruses, human herpes virus 6 and 7, parvovirus B19, human bocavirus, cytomegalovirus (whole blood), Epstein–Barr virus (whole blood), and JC, BK, WU, and KI polyomaviruses, all of which were negative. Quantitative PCR for AstV-MLB2 showed 4.5×10^5 copies of AstV-MLB2/ml of plasma.

Astrovirus-PS (closely related to AstV-VA1/HMOAstV-C) was found in the brain tissue of a 15-year-old boy with X-linked agammaglobulinemia and encephalitis by deep sequencing, quantitative PCR, and immunofluorescence staining [43]. The cerebral spinal fluid and brain material were negative for the common agents known to cause encephalitis. The histologic findings of the brain material were thought to be consistent with a viral infection.

While these case reports do not prove disease causality, they do suggest that astroviruses may have a role outside of the enteric system. These cases demonstrate a broader distribution of astroviruses in the body than previously recognized.

Nomenclature

With these exciting discoveries of new astroviruses come many challenges. One of these challenges is what to name these new viruses and how to decide if they represent new species. Previously, astroviruses were named for the species they infect (human, turkey, etc.). The classic human species consists of eight closely related serotypes which are named 1–8. In a similar fashion, the HMO astrovirus names are derived from their relationship to human, mink and ovine astroviruses. On the other hand, AstV-MLB1, AstV-VA1, and AstV-PS are named for the site that the sample originated from. A more uniform and instructive naming system needs to be put in place. Kapoor et al. have suggested a naming scheme in which human astroviruses 1–8 are renamed HAstV-1 with a letter (a–h) following the name to denote each serotype and then naming the novel astroviruses described in this chapter HAstV-2–HAstV [27]. This issue of nomenclature is currently being addressed by the International Committee on Taxonomy of Viruses (ICTV) study group for Astroviruses. Additionally, how to define a new species needs to be addressed. There are currently no specific rules in place.

Conclusions

Diarrhea is often not considered a major problem in the developed world, but it still has devastating effects in developing countries. There is a major gap in our understanding of the causes of diarrhea, given that ~40% of all cases are of unknown etiology [25, 33, 48]. While astroviruses are known to cause diarrhea, they have generally been associated with at most 10% of both sporadic and outbreak cases of acute diarrhea. The initial identification of five novel astroviruses in human stool samples over the course of a 2-year period hints that astroviruses may have a larger role in causing human diarrhea than previously believed. Prior to 2008, there were eight known human astroviruses that are all genetically very closely related. It is possible that this paradigm will be upheld for each of the newly described astroviruses in that each one could represent a whole new cluster of astroviruses. As technologies advance and more samples are screened from all over the world, it is likely that other novel astroviruses will be discovered.

The discovery of five novel astroviruses from human samples has nearly doubled the number of known human astroviruses. However, the identification of these novel viruses is surely only the beginning of the story for these viruses. There are many questions that have been raised regarding each virus' tropism, epidemiology, and potential link to disease. Answering these questions will require many years of further investigation, using the available tools of biology and medicine. While all of the recently discovered astroviruses described in this chapter were first identified in stool samples, some have now been detected in other biological specimens or in stools from people without diarrhea. These findings are in no way conclusive, but they do reveal the possibility that astroviruses may be associated with other diseases in humans. The major questions for all of these viruses are (1) Are they truly human pathogens? and (2) If so, what diseases do they cause? These questions are difficult to answer short of doing human infection trials. However, the studies described in this chapter have begun to address these questions.

Historically, the identification of a novel agent in association with a disease has been the rate-limiting step in addressing the question of disease causality. However, a shift has occurred in that the rate-limiting step often now comes after the discovery phase in trying to understand the biological relevance and impact of a newly discovered agent. Determining the role of novel human astroviruses in human health and disease will be an important focus of future efforts as the field of astroviruses moves forward.

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