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Perusal of parasitic nematode 'omics in the post-genomic era

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Abstract

The advent of high-throughput, next-generation sequencing methods combined with advances in computational biology and bioinformatics have greatly accelerated discovery within biomedical research. This “post-genomics” era has ushered in powerful approaches allowing one to quantify RNA transcript and protein abundance for every gene in the genome - often for multiple conditions. Herein, we chronicle how the post-genomics era has advanced our overall understanding of parasitic nematodes through transcriptomics and proteomics and highlight some of the important advances made in each major nematode clade. We primarily focus on organisms relevant to human health, given that nematode infections significantly impact disability-adjusted life years (DALY) scores within the developing world, but we also discuss organisms of veterinary importance as well as those used as laboratory models. As such, we envision that this review will serve as a comprehensive resource for those seeking a better understanding of basic parasitic nematode biology as well as those interested in targets for vaccination and pharmacological intervention.

INTRODUCTION

Parasitic helminths presently infect well over one billion people worldwide, and the number of individuals harboring one or more species may increase in the absence of therapeutic intervention. Among helminth parasites, the nematodes include multiple organisms with significant impact on human and animal health—including roundworms, hookworms, filarial worms, whipworms, and others [1]. Phylum Nematoda, which includes both free-living and parasitic species, has been divided into five main clades [2, 3] as well as 12 more specific clades [4]. Only clades containing nematodes that are parasites of vertebrate animals are discussed here (clades I, III, IV, and V), with a focus on species of medical and veterinary

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importance and their laboratory models. Within each clade, species are grouped and discussed by Family.

Over the past decade, advances in sequencing technology as well as bioinformatic capabilities have allowed an unprecedented number of helminth genomes to be sequenced [5]. While full genome sequences provide investigators with a “toolbox” for their organism of interest, the expression patterns and functions of these genes are often unknown. In recent years, investigators have begun leveraging genome sequences to interrogate the expression, localization, and function of every gene in the genome by utilizing whole transcriptome shotgun sequencing (RNAseq) and proteomics. RNAseq takes advantage of next-generation sequencing to quantify particular types of RNA transcripts, including polyadenylated RNA, small RNAs, or total RNA, which can be isolated from whole organisms, dissected tissues, or specific developmental stages. Advances in proteomic methods coupled with annotated genomes that can provide predicted protein sequences for every gene provide investigators with new capabilities to identify proteins in biological samples. The number of studies using these post-genomic ‘omic methods to investigate nematode parasitism has greatly increased since their most recent review [6]. Thus, the overarching goal of this review is to provide a broad overview of the most recent data.

Resources to navigate this data-rich environment are of incredible importance. Both NEMBASE (<http://www.nematodes.org/nembase4>) [7] and Nematode.net (<http://nematode.net>) [8] have cataloged many datasets for nematode parasites. Recently, WormBase ParaSite (<http://parasite.wormbase.org/>) has brought many features of WormBase to the parasite community [9]. The utility of these databases will undoubtedly increase as they are expanded and brought to bear on problems specific to parasitism.

CLADE I

The diverse nematodes found in clade I include parasites of vertebrates, invertebrates, and plants, as well as free-living species. From a human health perspective, *Trichuris trichiura* is perhaps the most important with approximately 600–800 million people currently infected worldwide [10]. *Trichinella spiralis* is also an important clade I pathogen that is transmitted via undercooked meat and infects approximately 10,000 people throughout the world each year [11].

Trichuridae

The life cycle of *Trichuris* spp., including *T. trichiura* (human), *T. muris* (mouse and rat), and *T. suis* (swine), involves adult female and male worms that reside in the large intestine, with their anterior ends buried in the mucosal epithelium [12]. *Trichuris* ova passed in host feces embryonate in soil and hatch upon ingestion, releasing first-stage larvae (L1) in the small intestine through a mechanism reliant on host microbiota [13]. Adult worms develop after four molts and subsequently reside within an intestinal epithelial syncytium in the host [12].

Given the importance of finding novel therapeutic targets, Foth *et al.* focused on the anterior portion of the parasite (the stichosome) that directly interfaces with the host. They found an

increased abundance of transcripts encoding many chymotrypsin A-like serine proteases, a gene family that is expanded in the *T. trichiura*, *T. muris*, and *T. suis* genomes [14, 15], that are known to degrade host intestinal mucins, and that may also serve digestive or immunomodulatory functions [14, 16]. Moreover, multiple DNase II-encoding transcripts with signal peptides were identified in the stichosome—indicating a possible function in degrading host DNA to dampen immune responses [14]. The mRNA and small RNAs expressed in the stichosome of *T. suis* encode a full complement of RNAi machinery as well as high levels of diverse peptidases and porins relative to adult worm posterior bodies. Curiously, 2/3 of miRNAs are most abundant in larval stages rather than in adults, with a significant decrease in the L3-L4 transition, suggestive of developmental regulation [15]. Consistent with evidence that trichurid nematodes may protect against allergic reactions, atopy, and autoimmune conditions [17], proteins such as fructose biphosphate aldolase and heat shock protein 70 have been identified as potential immunomodulatory agents [18, 19]. *Trichuris* spp. also inhibit neutrophil-mediated tissue remodeling via secreted chymotrypsin/elastase inhibitors [20] and release β -barrel toxins that perforate host cell membranes [14, 15].

Trichinellidae

Trichinella spiralis can infect a variety of vertebrate animals, including humans, swine, mice, and rats. The adult female and male worms, which reside inside intestinal epithelial cells, reproduce sexually, with females laying motile L1. These intestinal L1 transverse the gut epithelium, access the circulatory system, and invade striated muscle cells that become nurse cells. These muscle larvae undergo developmental arrest as L1, which are the infective form. Following consumption of infected muscle tissue, host digestive enzymes release L1 that rapidly reach the intestine, where they develop into adult worms after four larval molts [12].

New insights into the biology of *T. spiralis* indicate RNA-mediated gene regulation, with up to 45% of genes being transcribed from both strands [21]. Additionally, *T. spiralis* provided the first example of 5'-cytosine methylation in a nematode species; DNA methylation in the genome of this organism significantly increases during the transition from newborn L1 to muscle L1 [22]. RNA-directed DNA methylation and chromatin modification likely help control transposable elements and compensate for loss of piRNAs [23]. Furthermore, unlike other species, *T. spiralis* endo-siRNAs may be more important for gene regulation than suppression of transposable elements [24]. Newborn larvae that traverse the intestinal epithelium express multiple protease gene families, including astacin and serine proteases, and DNase II [21, 25]. Future comparisons of *Trichinella* spp. will be aided by genome sequences from each of the 12 recognized taxa [26].

Studies comparing proteomic profiles of excretory/secretory products (ESP) of *T. spiralis* and *T. pseudospiralis* as well as larval surface proteins involved in host-parasite interactions have identified multiple novel proteins [27, 28]. New antigens expressed across developmental stages, such as Ts 14-3-3, could be important for diagnosing trichinellosis in the context of co-infection with other parasitic nematodes, since co-infection often increases the incidence of false positives in tests [29]. Other somatic proteins, many of which are expressed on the parasite surface in muscle and intestinal forms of the parasite, may be

possible vaccination targets [30]. As might be expected, expression of genes encoding energy-generating proteins is up-regulated in intestinal infective larvae in comparison to muscle larvae [30].

CLADE III

Nematodes within this clade are all animal parasites, either of vertebrates or invertebrates, and a substantial proportion of them have a major impact on human health [32]. *Ascaris lumbricoides* likely has the largest impact in terms of DALYs, as 807–1,221 million people worldwide are infected with this species [10]. Multiple filarial worms infect humans and cause significant mortality, including *Brugia malayi* (~12 million), *Loa loa* (~13 million), *Onchocerca volvulus* (~37 million), and *Wuchereria bancrofti* (~108 million) [33, 34]. Additionally, the pinworm *Enterobius vermicularis* infects 4–28% of children worldwide [10], although it typically does not result in severe disease.

Ascarididae

While humans are the only definitive host for *A. lumbricoides*, infections with *A. suis* (swine) or *Toxocara canis* (canid) can cause significant human disease. Adult female and male worms live in the lumen of the small intestine, where gravid females produce up to 200,000 eggs per day [10]. Eggs passed in the feces embryonate in soil, and two molts occur within the egg before consumption by the host. Following ingestion, infective larvae emerge from the egg in the intestine, penetrate the gut wall, and enter circulation. Upon entering the pulmonary vasculature, the larvae penetrate the alveolar wall and are coughed up, making their way to the trachea and eventually returning to the intestine, where they molt twice more and mature into adult worms [12]. Most studies have focused on parasite stages relevant for initiating human disease.

Over the course of evolution, piRNAs, which are generally important for suppressing transposable elements in the germline, have been lost in *A. suum* and their function likely assumed by endo-siRNAs [35]. *Ascaris* spp. miRNAs appear to be synthesized immediately following fertilization [35], with targets in *A. lumbricoides* and *A. suum* including transcripts encoding ovarian message protein, vitellogenin, and chondroitin proteoglycan [36]. The *Ascaris* X chromosome undergoes diminution, a phenomenon observed in other parasitic nematodes, resulting in the loss of genes primarily expressed in the germline and during early embryogenesis [37]. Moreover, phosphorylation may regulate spermatogenesis, with an increase in transcripts encoding serine/threonine kinases and phosphatases during this process, in comparison to the somatic gonad [38]. The *Ascaris* ELT-2 transcription factor homolog likely controls development of the intestine, similar to its role in *Caenorhabditis elegans*. Furthermore, female parasites may have an ecdysone-mediated transcriptional network, while males may have an acetylcholine-mediated transcriptional network [39].

Transcripts encoding secreted peptidases with putative immunomodulatory activity are highly expressed within the *Ascaris* migratory larval stages, including *alt-1*, *cpi-2*, and *mif-4*; additionally, G protein-coupled receptors (GPCRs) may aid in chemotaxis associated with hepatopulmonary migration [40]. As expected, different life cycle stages present a

unique array of proteins to the host, with distinct profiles found in ESP versus peri-enteric and uterine fluid proteins [41]. Relative to ESP from egg- or lung-derived L3, ESP from *A. suum* L4 are enriched for metabolic enzymes, particularly glycosyl hydrolases, revealing the importance of host carbohydrate degradation for adult parasite metabolism [42]. In a related manner, *T. canis* adults are enriched with mRNA transcripts encoding lysosomal degradation and carbon metabolism proteins, while L3 are enriched for transcripts encoding neuronal signaling and cuticle formation/shedding proteins [43].

Onchocercidae

Lymphatic filarial worms include *Brugia* spp. and *W. bancrofti*, with adult females and males typically inhabiting host lymphatic vessels. The sheathed L1 (microfilariae) are ingested during a blood-meal by the mosquito intermediate host, molt twice, and migrate to the salivary glands where they developmentally arrest as infective L3. Infective L3 are transmitted to the definitive host via a subsequent blood meal and migrate to the lymphatic vessels, where they develop into adults [12].

The 46 collagen-encoding mRNA transcripts in *B. malayi* are highly regulated during development [44], and parasites residing within the *Aedes aegypti* host show transcriptomic profiles reflective of the molting process [45]. During early infection of the mosquito host, transcripts encoding proteases and stress-response proteins are increased, in comparison to microfilariae [45]. While no evidence of DNA methylation or piRNAs was found in *B. malayi*, the identified siRNAs likely control transposable elements during development [23]. In *B. pahangi*, most miRNAs are novel, with only 42 of 104 conserved in other organisms at the time that report was published [46]. Other work has found that 20-hydroxyecdysone treatment results in increased transcript abundance for genes involved in embryogenesis and may regulate egg development, similar to its regulatory role in insects [47].

Proteomic studies have identified the glycolytic enzyme triose phosphate isomerase, and other metabolic enzymes, in large quantities in ESP of *B. malayi* [48, 49]. Triose phosphate isomerase could be one of the immunomodulatory proteins that induces alternative macrophage activation in infected hosts. Further stage-specific proteomics confirmed the presence of 47% of the proteins predicted in the draft genome [50]. Analysis of three anatomic fractions (body wall, digestive, and reproductive tract) revealed 27 *B. malayi* proteins, with high homology to *W. bancrofti* and *O. volvulus*, as possible vaccine candidates [51].

Additional filarial worms, including *Dirofilaria* spp., *Litomosoides* spp., *L. loa*, and *Onchocerca* spp., have life cycles similar to lymphatic filariae but with different arthropod vectors and tissue specificities for the adult worms. Adult female and male *D. immitis*, a parasite of canids and felids that is commonly referred to as heartworm, are found in the pulmonary arteries and heart chambers, with mosquitoes acting as intermediate hosts and vectors [12]. Among filarial nematodes, studies of *D. immitis* transcriptomics and proteomics benefit from the large size of this organism, which allows ease of micro-dissection. Studies focused on *D. immitis* have identified lists of genes possibly involved in cardiac pathogenicity [52, 53]. The transcriptomes derived from whole adult female or male heartworms are dominated by transcripts from the reproductive tissue [54]. While most *D.*

immitis miRNAs are conserved with *B. malayi*, 13 novel miRNAs have been identified [55]. Biosynthesis of heme by the *Wolbachia* endosymbiont predominantly occurs in microfilariae and corresponds with expression of *D. immitis* heme-binding proteins [56]. Studies of *D. immitis* ESP [57], as well as somatic and surface proteins [58], have identified multiple proteins involved in host-parasite interactions. Further studies of *D. immitis*-infected tissue identified up to 32 % of the predicted *D. immitis* proteome, including the detection of >700 hypothetical proteins [54].

L. sigmodontis, a parasite of rats and a widely used experimental model, employs mites as its intermediate host; adult female and male worms are found in the pleural cavity of the definitive host [12]. An RNAseq dataset exists but has not been extensively interrogated [59]. Proteomics data across several life stages reveal transthyretin-like proteins in all developmental stages examined, as well as the identification of 302 ESP—4 of which may be novel vaccine candidates [60].

Humans are the definitive host for *L. loa*, for which day-biting flies such as *Crysops* spp. are the intermediate hosts, with adult females and males being found in the subcutaneous connective tissues [12]. Studies of putative biomarkers for *L. loa* microfilariae in infected host urine identified 18 proteins specific to microfilariae; two of these were immunogenic *in vivo*. Additionally, LOAG_16297 was described as a biomarker that can distinguish between infection with *L. loa* and other microfilariae [61].

Microfilariae of *Onchocerca* spp. are unsheathed and typically found in the skin, rather than the blood. The human parasite *O. volvulus* is transmitted by black flies; microfilariae of *O. volvulus* frequently invade the eyes and can cause blindness. The adult female and male worms typically reside in nodules in subcutaneous tissue [12]. In contrast to *O. volvulus* and other filaria, *O. flexuosa* is *Wolbachia*-free, and studies have leveraged this to identify several unique genes expressed in *O. flexuosa* that are only found in the *Wolbachia* within *B. malayi*, not the *B. malayi* genome [62]. Proteomics revealed comparatively higher levels of mitochondrial-related protein families in larval stages and a preponderance of antimicrobial proteins, especially cathelicidins, in the nodule fluid, in a study that also included adult worms [63].

CLADE IV

While clade IV contains a diverse set of nematodes, including free-living species as well as plant and insect parasites, the species that infect vertebrates are almost entirely limited to the family *Strongyloididae* [2]. Over 52 *Strongyloides* spp. have been documented, with each species having a narrow vertebrate host range [68]. The species of greatest concern for human health is *S. stercoralis*, with approximately 30–100 million people infected globally [10].

Strongyloididae

Of the nematode parasites, *Strongyloides* spp. are unique in having parthenogenetic parasitic females that reside in the lumen of the intestine and pass either eggs or L1 in the feces of the host. Outside the host, female larvae can develop via two routes: a homogonic route, where

larvae progress through two molts to become infective L3, or a heterogonic route, where larvae progress through four molts to become free-living adults. Male larvae develop exclusively to free-living adults. In most *Strongyloides* spp., the free-living generation of females and males produces a post-free-living generation consisting entirely of female larvae that invariably develop to infective L3. Similar to other soil-transmitted nematodes, these developmentally arrested L3 can penetrate the skin of their respective hosts and, through a complex internal route, make their way to the intestine where they develop to the adult parasitic female. Unique to *S. stercoralis* is the ability of larvae to develop precociously to autoinfective L3 within the primary host and establish a second generation of parasitic females. Particularly in immunocompromised hosts, successive waves of autoinfection may cause a fatal condition known as hyperinfection [68].

Recent transcriptomic studies in *S. stercoralis*, *S. ratti*, and *S. venezuelensis* have highlighted an expansion of astacin and SCP/TAPS genes, which encode metalloproteases and putative immunomodulatory proteins, in comparison to *Rhabditophanes* sp., their closest free-living relatives [69]. *Strongyloides* spp. astacin and SCP/TAPS transcripts are particularly abundant in parasitic females. Moreover, many parasitism-associated genes are found in co-regulated clusters. In comparison to parasitic females, many genes up-regulated in infective L3 encode proteins involved in sensing environmental cues and signal transduction [69]. Additional work has identified transcripts in infective larvae [70], including differentially expressed astacins during L3 migration [71]. *S. stercoralis* transcripts encoding homologs of cGMP and TGF β signaling proteins that regulate *C. elegans* dauer development are coordinately increased in infective larvae, and several insulin-like peptide-encoding transcripts are specifically regulated throughout the life cycle [72]. A putative pathway for *in vivo* production of dafachronic acid that regulates *S. stercoralis* development has been identified [72, 73], and dafachronic acid transcriptionally recapitulates *in vivo* activation of infective larvae [74]. The possibility of manipulating this crucial developmental regulatory pathway with administered small molecules underscores its potential as a chemotherapeutic target. In *S. ratti*, miRNAs homologous to those regulating dauer arrest in *C. elegans* also regulate development in the parasite [75]. Proteomics work in *S. ratti* identified an abundance of stage-specific proteins in infective larvae, parasitic females, and free-living stages [69, 76], which may prove valuable as future drug or vaccine targets.

CLADE V

Diverse nematodes are represented in clade V, including parasitic and free-living species. Notably, the intensively studied bacterivore *C. elegans* is a member of this clade [2]. Many vertebrate parasites in clade V develop similarly to *C. elegans* dauer constitutive mutants, with the infective larvae arresting as L3 [77]. Important constituents of this clade are the hookworm species that infect an estimated 576–740 million people worldwide, causing severe anemia and malnutrition [10]. Other members of clade V are the strongyles and trichostrongyles, which include several parasites that significantly impact animal agriculture and welfare. The rodent-infecting nematodes *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis*, which are widely used as laboratory models for immunological research, are members of this clade and have life cycles that resemble those of the trichostrongyles and hookworms, respectively.

Ancylostomatidae

The vast majority of human hookworm infections are caused by *Ancylostoma duodenale*, *A. ceylanicum*, and *Necator americanus*. Additional hookworm species can infect other animals, including *A. caninum* (canines). These hematophagous species have direct life cycles, with females and males feeding off the capillary beds in the small intestine. Females lay eggs that are passed in the feces and embryonate outside the host. In the soil, L1 emerge from the egg and mature through two molts to the infective L3, which are sheathed, non-feeding, and developmentally arrested. L3 enter a permissive host by direct skin penetration, travel to the heart and then the lungs via the circulatory system, and molt to the L4, which are coughed up and swallowed. Maturation to the adult then occurs in the intestine [12].

In infective L3, transcripts encoding GPCRs, signal transduction, and ion channel proteins are abundant—consistent with the necessity to sense and transduce host cues [78, 79]. These transcripts decrease once larvae enter the host, while transcripts encoding cysteine-rich activation-associated secreted proteins that likely block blood clotting are increased [78]. In comparison to larval stages, transcripts encoding C-type lectins and secreted proteins are up-regulated in adult worms [78, 80], a finding consistent with the abundance of proteases and lectins in ESP [80]. In comparison to infective larvae, adults also have abundant transcripts for hydrolases, catalases, and single-domain serine protease inhibitors that may function as anticoagulants and immunomodulatory proteins. Similar to *Strongyloides* spp., the number of hookworm genes encoding SCP/TAPS proteins is greatly increased in comparison to its closest well-studied free-living relative, *C. elegans* [79]. Lists of parasite-associated genes may aid in future therapies [81, 82].

Trichostrongylidae

The trichostrongyles, including *Cooperia oncophora*, *Haemonchus contortus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta*, and *Trichostrongylus colubriformis*, have similar life cycles and cause significant disease in livestock. *H. contortus* are unique in that they feed by blood-sucking. Adult female and male worms reside in the gastrointestinal tract, either in the small intestine (*C. oncophora* & *T. colubriformis*) or abomasum (*H. contortus*, *O. ostertagi*, & *T. circumcincta*). Generally, eggs are excreted in the feces of the infected host and hatched L1 develop to sheathed L3, the infective form. In contrast to active skin-penetrating infective L3 of hookworms, the stress-resistant and developmentally arrested infective L3 of trichostrongyles migrate to the tips of grass blades, where they are passively ingested by grazing herbivores. The L3 typically invade gastric tissue where they undergo two molts before returning to the lumen to complete development into reproductive adults. For several species that infect hosts at higher latitudes, larvae can arrest as L4 within gastric glands until the following spring [12].

Larval stages of *C. oncophora* have abundant transcripts encoding MADF transcription factors and chromo domains potentially involved in germline and vulval development. In contrast, adult parasites have abundant transcripts for zinc-finger transcription factors and CAP domains [83]. Proteomic work has identified a non-glycosylated protein as a potential vaccine candidate [84].

Changes in mRNA transcript abundance throughout the *H. contortus* life cycle have been carefully examined. Transcripts encoding proteins involved in muscle development increase as the worm develops from egg to L1, while transcripts encoding metabolic enzymes decrease. During developmental arrest as L3, transcripts encoding proteins involved in O₂ transport and heme-binding, as well as transcripts encoding cytochrome P450s, increase [85], as do transcripts encoding channels, GPCRs, and signaling components [86]. During exsheathment, peptidase-encoding transcripts are abundant [87]. The L3 to L4 transition is accompanied by an increase in transcripts for proteins involved in movement and metabolic activity, while the L4 to adult molt correlates with an increase in transcripts for proteins found in reproductive tissues [85]. In blood-feeding adults, transcripts encoding collagens, SCP/TAPS, and peptidases for tissue degradation become more abundant [86]. A study of small RNAs in *H. contortus* adults revealed piRNAs that may be involved in germline maintenance [46]. Congruent with the emergence of drug resistance in *H. contortus*, a comparative analysis of the proteome from ivermectin-resistant and -susceptible worms showed an increased proportion of proteins involved in metabolic detoxification in the resistant isolate [88]. Additional proteomics work has identified potential vaccine candidates [89, 90].

There is considerable interest in developing new approaches to exploit natural immunity to *T. circumcincta* in sheep to reduce worm burden. Possible vaccine candidates have been identified in ESP, including transcripts encoding SCP-like extracellular proteins, C-type lectins, saposin-like proteins, and allergen V5/Tpx-1-related proteins [91]. Several vaccine candidates have also been identified in the ESP of post-infective larvae [92], including *Tci*-ASP-1, which is present in L4 and adult stages. Purified recombinant *Tci*-ASP-1 is recognized by IgA [93]. Extracellular vesicles in the ESP of *T. circumcincta* have a high percentage of unique proteins that are targets of both IgA- and IgG-mediated responses, and which may be potential targets for future vaccine studies [94].

Strongylidae

Adults of the swine parasite *Oesophagostomum dentatum* reside in the large intestine. Eggs develop outside the host to infective L3, which are sheathed. Inside the host, L3 invade intestinal mucosa, complete two larval molts, and re-enter the lumen as adults. Members of the genus *Strongylus* and genera within the subfamily Cyathostominae are also important pathogens of horses [12]. *O. dentatum* transcripts encode proteins associated with the targets of benzimidazole, macrocyclic lactone, and nicotinic agonist classes of anthelmintic drugs [95]. Proteomic work identified several proteins down-regulated during hydrolase inhibitor-mediated developmental arrest [96], as well as 11 proteins hypothesized to be involved in exsheathment [97].

Dictyocaulidae

The adults of *Dictyocaulus* spp. live in the lungs—giving rise to the term lungworms. These trichostrongyles are not to be confused with members of the superfamily Metastrongyloidea that are also commonly called lungworms. L1 of *Dictyocaulus* hatch in air passages and, after being passed in the feces, develop to infective L3 in the environment. Developmentally arrested L3, which may be aided in their dispersal by exploding sporangia of *Pilobolus* spp.

fungi, are ingested on herbage. Inside the host, they penetrate the intestinal wall and migrate to the lymph nodes where they complete two molts, then travel through the lymphatic system to the heart and eventually the lungs [12]. *D. filaria* (ruminants) and *D. viviparus* (bovine) express multiple transcripts encoding proteins such as secreted chymotrypsin-like proteases, SCP/TAPS proteins, and others that may be involved in host-parasite interactions [98, 99]. In *D. viviparus* free-living stages, transcripts encoding proteins involved in the regulation of transcription, GPCR and steroid hormone-mediated signaling, and the transport of ions and oxygen are over-represented. In parasitic stages, transcripts encoding proteins involved in protein translation, redox homeostasis, and energy metabolism are over-represented. Potential new drug targets have been identified in *D. viviparus*, including carboxylesterase and a *C. elegans* ACE-3 homolog [99].

Heligmonellidae

Both *H. polygyrus* and *N. brasiliensis* adults reside in the small intestine, but *N. brasiliensis* has a life cycle similar to many hookworms in that soil-resident infective L3 penetrate skin, while *H. polygyrus* infective L3 are only transmitted through passive ingestion. *N. brasiliensis* L3 exsheath after host invasion and then migrate to the lungs, before being swallowed to reach the intestine and mature to adults, which are expelled in 1–2 weeks. In contrast, *H. polygyrus* L3 penetrate the intestinal mucosa, complete two molts, and then return to the lumen where the adults feed on host tissues [12].

Efforts to examine host-parasite interactions utilizing *H. polygyrus bakeri* have largely focused on ESP. The more than 200 known *H. polygyrus* ESP include possible immunomodulators [100] and proteins similar to ESP of other parasitic nematodes [101]. Interestingly, *H. polygyrus bakeri* also secretes vesicles (exosomes) containing miRNAs and Y RNAs that suppress Th2 immune responses and eosinophilia [102]. In *N. brasiliensis*, astacin metalloproteases and CAP-domain-containing SCP/TAPS are abundant in both L3 and adult ESP, though multiple ESP are stage specific [103]. Additionally, 21U RNAs (piRNAs) are present in *N. brasiliensis*, as well as *H. contortus* and *C. elegans*, suggesting conservation across clade V nematodes [23].

Angiostrongylidae

Angiostrongylus spp. adults are typically found in the pulmonary arteries and utilize an intermediate host, a rare feature among clade V parasitic nematodes. Eggs in the bloodstream become lodged in the lungs where they embryonate; L1 are then excreted in the feces and infect a variety of gastropods. When ingested, larvae then migrate to the brain (*A. cantonensis*) or lymph nodes (*A. vasorum*), where they develop to the adult form, before eventually re-entering circulation and migrating to the heart. While rats are the definitive host for *A. cantonensis*, this parasite can also infect humans as a zoonosis.

Angiostrongylus spp. adults express high levels of mRNA transcripts that encode proteins involved in hemoglobin digestion and predicted immunogenic ESP [104]. Homology-based searches from *C. elegans* RNAi phenotypes have also been used to identify potential drug targets for these organisms [104]. An array of stage-specific proteins in *Angiostrongylus* spp. have now been identified [105], some of which are highly immunogenic proteins that

could be used for diagnostic assays [106, 107]. Brain-derived young adults contain a variety of mRNA transcripts encoding heat shock proteins, oxidative stress proteins, and proteases [108]; proteomic differences between L3 and L5 stages are also present in snails and rats, respectively [109].

DISCUSSION

Shared features of many vertebrate parasites, such as developmental arrest at specific larval stages and secretion of proteases, may be a result of intrinsic nematode developmental biology that seemingly aided the evolution of parasitism, gene expansions that have occurred in multiple lineages, or convergent evolution. Parasitic nematode ‘omic studies have uncovered multiple commonalities of this group of organisms. Invasive and tissue-migratory stages often secrete a variety of proteases, and genomic studies have revealed expansions of gene families encoding immunomodulatory proteins in multiple parasitic nematode clades. Transcriptomic studies of infective larvae that reside in the environment have revealed an up-regulation of transcripts encoding proteins involved in environmental signal recognition and transduction pathways, particularly GPCR signaling. Additionally, piRNA-encoding genes appear to have been lost in several parasitic nematode lineages, with their function in suppressing germline transposons having been subsumed by other processes and small RNAs in these groups of organisms. Proteomic studies of parasite ESP have identified various effector proteins that may dampen host immune responses, protect a parasitic niche, and aid in parasite migration and feeding inside the host.

While there are many shared features in parasitic nematodes, some strategies for parasitism may be unique within specific clades. For example, in several clade I nematodes, secreted DNase II has been observed and is postulated to have immunomodulatory effects in the host. Furthermore, 5'-cytosine methylation of *Trichuris* DNA may be unique, as no evidence has been found for this process in *B. malayi* or *C. elegans* [23]; however, adenine N⁶-methylation has recently been described in *C. elegans* and may be found in other parasitic nematode lineages [113]. To date, ecdysone-mediated transcriptional networks have only been observed in the clade III *Ascaris* and *Brugia* spp., though future work may expand this observation to other parasite lineages. While the mechanisms regulating the unique life cycle of *Strongyloides* spp. remain enigmatic, genes in pathways homologous to those that control *C. elegans* dauer development appear to have assumed parasite-specific functions, such as host-sensing, in this group. Interestingly, some clade V nematodes, such as *H. polygyrus bakeri*, are capable of secreting exosomes containing immunomodulatory miRNAs and Y RNAs. While these observations may currently be limited to select nematodes, future studies will almost certainly illuminate whether such mechanisms are more broadly shared.

One of the shortcomings of ‘omic projects is the tendency to focus on previously characterized genes, while ignoring the significant percentage of uncharacterized or hypothetical genes present in a parasite’s genome. Parasitic nematodes clearly have unique biology in comparison to their free-living counterparts, which we believe may, in part, be regulated by these “novel” genes. Thus, the focus on genes with orthologs in well characterized free-living organisms, such as *C. elegans*, may result in overlooking the key factors regulating nematode parasitism. We recognize that characterizing genes unique to

parasitic nematodes is a challenge, particularly on a genome-scale. However, we note several strategies that can be taken to address this. First, experiments should be designed such that comparisons between specific conditions are biologically informative, with enough biological replicates to draw meaningful conclusions, and that all genes in the genome are considered for regulating observed differences. This is an underutilized strength of many post-genomic ‘omic methods, which allow comparisons for both well-characterized and novel genes. Second, the establishment of phylogenetically relevant free-living nematode models may help elucidate the key regulators of vertebrate parasitism. While convenient and particularly well-studied, *C. elegans* may not be the appropriate comparison for many parasitic nematodes given the divergent evolutionary histories of each clade of parasitic nematodes discussed here. These and other methods will almost certainly help researchers begin to uncover the genetic basis of nematode parasitism.

In this review, we have provided a summary of the insights gained from post-genomic ‘omics technology applied to nematodes. Given the profound and continuing importance of parasitic nematodes in human and veterinary medicine, it is likely that the continued accumulation of data in this realm will lead to breakthroughs in the design of novel drugs and vaccines targeting these parasites. While the rate of advances in the molecular biology of these organisms has historically lagged behind other more genetically tractable phyla, the introduction of deep sequencing and advanced proteomic data mining is rapidly closing the gap. Perhaps with the advent of CRISPR/CAS9 targeting, along with the complete sequencing of additional parasitic and free-living nematode genomes, there will be an even greater acceleration of scientific discovery in the near future for these largely enigmatic organisms. Moreover, we would suggest that new additions to the ongoing databases include accession numbers for raw data, which will make mining these valuable resources more amenable for future investigators.

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Highlights

- Review of post-genomic ‘omic studies in parasitic nematodes of vertebrates
- Focus on transcriptomic and proteomic studies examining basic parasite biology and treatment strategies
- Recommendations for future parasitic nematode ‘omic studies

Table 1

Selected post-genomic studies of clade I nematodes [2].

Species	Clade [4]	Host	Life stage(s)	Platform / Method	Accession	Reference
mRNAs						
<i>Trichinella spiralis</i>	2A	Human, Swine	newborn larvae, muscle larvae, adult	Illumina Solexa	GEO: GSE39151	[21]
			newborn larvae, muscle larvae, adult	Illumina HiSeq2000	GEO: GSE39328	[22]
<i>Trichuris muris</i>	2A	Mouse	L2, L3, adult female, adult male, female and male tissues	Illumina HiSeq2000	AE: E-ERAD-125	[14]
<i>Trichuris suis</i>	2A	Swine	adult	Illumina GAII	n/a	[31]
			L1/L2, L3, L4, adult female, adult male, female and male tissues	Illumina HiSeq2000	ENA: PRJNA208415	[15]
<i>Trichuris trichiura</i>	2A	Human	adult	Ion Torrent Ion 318	ENA: PRJEB12315	[19]
Small RNAs						
<i>Trichinella spiralis</i>	2A	Human, Swine	newborn larvae, muscle larvae, adult	Illumina Solexa	n/a	[24]
			L1 (muscle), adult	Illumina MiSeq	GEO: GSE56651	[23]
<i>Trichuris suis</i>	2A	Swine	L1/L2, L3, L4, adult female, adult male, adult female and male tissues	Illumina GAII	ENA: PRJNA208415	[15]
Methylation						
<i>Trichinella spiralis</i>	2A	Human, Swine	newborn larvae, muscle larvae, adult	Illumina HiSeq2000	GEO: GSE39328	[22]
Proteins						
<i>Trichinella spiralis</i>	2A	Human, Swine	ESP from muscle larvae	PB MALDI-TOF-TOF, MS/MS		[27]
			ESP from muscle larvae	AB Sciex 4800 MALDI-TOF/TOF-MS		[25]
			surface protein from muscle larvae	AB Sciex 4800 MALDI-TOF MS		[28]
			adult somatic proteins	AB Sciex 4800 MALDI-TOF/TOF-MS		[29]
			surface protein from muscle and intestinal infective larvae	n/a		[30]
<i>Trichuris trichiura</i>	2A	Human	adult somatic extract	W Micromass Q-TOF micro MS		[18]

Abbreviations: Applied Biosystems (AB); European Bioinformatics Institute (EBI); EBI ArrayExpress (AE); EBI European Nucleotide Archive (ENA); excretory/secretory products (ESP); mass spectrometer (MS); matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF); National Center for Biotechnology Information (NCBI); NCBI Gene Expression Omnibus (GEO); PerSpective Biosystems (PB); Waters (W)

Table 2

Selected post-genomic studies of clade III nematodes [2].

Species	Clade [4]	Host	Life stage(s)	Platform / Method	Accession	Reference
mRNAs						
<i>Anguillicola crassus</i>	8A	Eel	L2, adult female, adult male	Roche 454 GS FLX	SRA: SRP010313	[64]
			geographical isolates	Illumina GAIIX	SRA: SRP010313	[65]
<i>Ascaris suum</i>	8B	Swine	egg L3, liver L3, lung L3, L4, adult female, adult male	Illumina HiSeq2000	SRA: SRP010159	[40]
			adult male tissues	Roche 454	n/a	[38]
			embryo, larvae, adult tissues	Illumina GAIIX	GEO: GSE26956	[35]
			embryo, larvae, adult tissues	Illumina GAIIX	GEO: GSE38470	[37]
			reproductive & non-reproductive adult tissues	Illumina HiSeq2000	SRA: SRP013609	[39]
<i>Brugia malayi</i>	8B	Human	egg, microfilaria, L3, L4, adult female, adult male	Illumina GAIIX	AE: E-MTAB-811	[44]
			intra-mosquito host	Illumina GAIIX	GEO: GSE53664	[45]
			drug-treated adult female	Illumina MiSeq	SRA: SRP064921	[47]
<i>Brugia pahangi</i>	8B	Canine, Feline	microfilaria, L3 stages, L4, adult female, adult male	Illumina HiSeq2000	ENA: ERP001375	WTSI
<i>Diriofilaria immitis</i>	8B	Canine	adult female, adult male	Illumina GAIIX	AE: E-MTAB-714	[52]
			adult	Illumina HiSeq2000	SRA: SRA048975	[53]
			microfilaria, L3, L4, adult female, adult male	Illumina GAIIX	SRA: SRP048819	[56]
			adult tissues	Illumina GAIIX	GEO: GSE67894	[54]
<i>Litomosoides sigmodontis</i>	8B	Mouse	microfilaria, adult female, adult male	Roche 454 GS FLX	SRA: ERA011678	[59]
<i>Loa loa</i>	8B	Human	n/a	Illumina GAIIX	BP: PRJNA60555	BI
<i>Onchocerca flexuosa</i>	8B	Deer	mixed stages	Roche 454 GS FLX	BP: PRJNA65265	[62]
<i>Onchocerca volvulus</i>	8B	Human	microfilaria, L2, L3, adult female	Illumina HiSeq2000	ENA: ERP001350	WTSI
<i>Toxocara canis</i>	8B	Canine, Human zoonosis	L3, adult female, adult male, adult tissues	Illumina GAIIX	SRA: SRP051082	[43]
Small RNAs						
<i>Ascaris lumbricoides</i>	8B	Human	miRNAs from parasitic adult	Illumina Solexa	n/a	[36]
<i>Ascaris suum</i>	8B	Swine	miRNAs from parasitic adult	Illumina Solexa	n/a	[36]
			egg, early embryo, L1, L2, adult tissues	Illumina GAIIX	GEO: GSE26957	[35]
<i>Brugia malayi</i>	8B	Human	adult	Illumina MiSeq	GEO: GSE56651	[23]

Species	Clade [4]	Host	Life stage(s)	Platform / Method	Accession	Reference
<i>Brugia pahangi</i>	8B	Canine, Feline	L3, adult	Illumina GAI	SRA: SRP009892	[46]
<i>Dirofilaria immitis</i>	8B	Canine	adult	Illumina Solexa	GEO: GSE35646	[55]
Proteins						
<i>Ascaris suum</i>	8B	Swine	ESP from egg L3, lung L3, L4	W ESI Q-TOF Premier MS		[42]
			ESP, perienteric and uterine fluids from adult female	TF LTQ-Orbitrap Elite MS		[41]
<i>Brugia malayi</i>	8B	Human	ESP from adult	AB 4700 Proteomics Analyzer MS		[48]
			ESP from microfilaria, adult female, adult male	W Micromass Q-TOF micro MS		[66]
			ESP from microfilaria, infective L3, molting L3, adult female, adult male	linear ion trap-FT MS		[49]
			tissue from L3, adult female, adult male; blood-borne and uterine microfilaria	linear ion trap-FT MS		[50]
			adult female tissues	TFS LTQ-Orbitrap XL MS		[51]
			drug-treated adult female	n/a		[47]
<i>Brugia pahangi</i>	8B	Canine, Feline	adult somatic extracts	AB Proteomics 4700 MALDI-TOF-TOF Analyzer MS		[67]
<i>Dirofilaria immitis</i>	8B	Canine	ESP from adult	TF LTQ-FT Ultra Mass MS		[57]
			adult somatic & surface antigens	AB QSTAR-XL nanoESI-Q-TOF MS		[58]
			adult tissues	TFS QExactive MS		[54]
<i>Litomosoides sigmodontis</i>	8B	Mouse	ESP and tissue from adult female and adult male	TFS LTQ-Orbitrap Velos MS		[60]
<i>Loa loa</i>	8B	Human	urine from infected human	TFS LTQ-Orbitrap Velos MS		[61]
<i>Onchocerca flexuosa</i>	8B	Deer	adult lysates	TFS LTQ Orbitrap Discovery hybrid MS		[62]
<i>Onchocerca ochengi</i>	8B	Bovine	L3, adult female, adult male, uterine microfilaria	TFS LTQ-Orbitrap Velos MS		[63]

Abbreviations: Broad Institute (BI); European Bioinformatics Institute (EBI); EBI ArrayExpress (AE); EBI European Nucleotide Archive (ENA); excretory/secretory products (ESP); mass spectrometer (MS); matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF); National Center for Biotechnology Information (NCBI); NCBI BioProject (BP); NCBI Gene Expression Omnibus (GEO); NCBI Sequence Read Archive (SRA); Thermo-Fisher/ThermoFisher Scientific (TF/TFS); Waters (W); Wellcome Trust Sanger Institute (WTSI)

Table 3

Selected post-genomic studies of clade IV nematodes [2].

Species	Clade [4]	Host	Life stage(s)	Platform / Method	Accession	Reference
mRNAs						
<i>Strongyloides ratti</i>	10B	Rat	infective L3, free-living female, parasitic female	Illumina HiSeq2000, GAII, MiSeq	ENA: ERP002187, ERP001672	[69]
<i>Strongyloides stercoralis</i>	10B	Human, Canine	infective L3	Roche 454 GS FLX	SRA: ERP000798	[70]
			post-parasitic L1 & L3, free-living female, post-free-living L1, infective L3, activated L3, parasitic female	Illumina HiSeq2000	AE: E-MTAB-1164	[72]
			drug-treated infective L3	Illumina HiSeq2000	AE: E-MTAB-2192	[74]
<i>Strongyloides venezuelensis</i>	10B	Rat	egg/L1, infective L3, induced L3, parasitic female	Roche 454 GS FLX	SRA: DRA000395	[71]
			egg, L1, infective L3, induced L3, free-living female, parasitic female	Illumina HiSeq2000	BP: PRJDB3457	[69]
Small RNAs						
<i>Strongyloides ratti</i>	10B	Rat	infective L3, mixed stages	Illumina GAII	GEO: GSE41402	[75]
Proteins						
<i>Strongyloides ratti</i>	10B	Rat	ESP from infective L3, parasitic female, free-living female	TS LTQ MS		[76]
			parasitic female, free-living female	TFS LTQ-Orbitrap Velos MS		[69]

Abbreviations: European Bioinformatics Institute (EBI); EBI ArrayExpress (AE); EBI European Nucleotide Archive (ENA); excretory/secretory products (ESP); mass spectrometer (MS); National Center for Biotechnology Information (NCBI); NCBI BioProject (BP); NCBI Gene Expression Omnibus (GEO); NCBI Sequence Read Archive (SRA); Thermo-Fisher/ThermoFisher Scientific (TF/TFS)

Table 4

Selected post-genomic studies of clade V nematodes [2].

Species	Clade [4]	Host	Life stage(s)	Platform / Method	Accession	Reference
mRNAs						
<i>Ancylostoma ceylanicum</i>	9B	Canine, Feline, Human zoonosis	infective larva, L4, adult, drug-treated adult	Illumina HiSeq2000	BP: PRJNA231490	[78]
<i>Ancylostoma caninum</i>	9B	Canine	infective L3, serum-stimulated L3, adult male, adult female	Roche 454 GS FLX	BP: PRJNA20441	[81]
<i>Angiostrongylus cantonensis</i>	9C	Rat, Human zoonosis	L5	Illumina Solexa	n/a	[108]
<i>Angiostrongylus vasorum</i>	9C	Canine	L5, adult female	Illumina HiSeq2500	BP: PRJNA314004	XU
<i>Cooperia oncophora</i>	9B	Ruminant	adult	Illumina GAII	n/a	[104]
			egg, L1, L2, L3 sheathed, L3 exsheathed, L4, adult female, adult male	Roche 454 GS FLX	ENA: PRJNA72571	[83]
<i>Cylicostephanus goldi</i>	9B	Horse	adult	Roche 454 GS FLX	ENA: PRJEB3962	[110]
<i>Dictyocaulus filaria</i>	9B	Ruminant	mixed sex adult	Illumina GAII	SRA: SRP032224	[98]
<i>Dictyocaulus viviparus</i>	9B	Bovine	mixed sex adult	Illumina HiSeq2000	SRA: SRR1021571, SRR1021573	[98]
			egg, L1, L2, L3, L4, L5 female, L5 male, adult female, adult male	Illumina HiSeq2000	BP: PRJNA72587	[99]
<i>Haemonchus contortus</i>	9B	Ruminant	L3 sheathed, L3 exsheathed	Roche 454 GS FLX	n/a	[87]
			adult	Illumina GA II	n/a	[111]
			egg, L1, L2, L3, L4, adult female, adult male	Illumina GA II	SRA: SRP026668	[86]
			egg, L1, L3, L4, adult female, adult male, adult tissues	Illumina HiSeq2000	ENA: PRJEB1360	[85]
<i>Heligmosomoides polygyrus (bakeri)</i>	9B	Mouse	adult female, adult male	Illumina GA II	n/a	[100]
<i>Necator americanus</i>	9B	Human	adult	Roche 454 GS FLX	n/a	[82]
			L3, adult	Roche 454 GS FLX	BP: PRJNA72135	[79]
<i>Nippostrongylus brasiliensis</i>	9B	Rat	egg, L3, L4, adult	Illumina GA II	n/a	[103]
<i>Oesophagostomum dentatum</i>	9B	Swine	adult female, adult male	Illumina GA IIx	GB: SRR393668, SRR393669	[95]
<i>Ostertagia ostertagi</i>	9B	Bovine	egg, L1, L2, sheathed L3, exsheathed L3, L4, adult	Roche 454 GS FLX	ENA: PRJNA72577	[83]
<i>Teladorsagia (Ostertagia) circumcincta</i>	9B	Sheep, Ruminant	adult	Roche 454 GS FLX	SRA: SRR328404, SRR328405	[91]

Species	Clade [4]	Host	Life stage(s)	Platform / Method	Accession	Reference
<i>Trichostrongylus colubriformis</i>	9B	Sheep	adult female, adult male	Roche 454 GS FLX	SRA: SRP002574	[112]
Small RNAs						
<i>Haemonchus contortus</i>	9B	Ruminant	sheathed L3, adult	Illumina GA II	BP: PRJNA151375	[46]
<i>Heligmosomoides polygyrus (bakert)</i>	9B	Mouse	egg, L3, adult	Illumina GA IIx	GEO: GSE55978	[102]
<i>Nippostrongylus brasiliensis</i>	9B	Rat	adult	Illumina MiSeq	GEO: GSE56651	[23]
Proteins						
<i>Ancylostoma caninum</i>	9B	Canine	ESP from adult	BD micrOTOF-Q		[80]
<i>Angiostrongylus cantonensis</i>	9C	Rat, Human zoonosis	ESP from adult female	BD maXis ESI-Q-TOF MS		[107]
			tissue from L3, female L5, male L5, adult female, adult male	AB 4800 MALDI-TOF/TOF MS		[105]
			L3, L5	BD Ultraflex MALDI-TOF MS		[109]
<i>Angiostrongylus costaricensis</i>	9C	Rat, Human	tissue from adult female, adult male	ABI Sciex 5800 MALDI-TOF/TOF MS		[106]
<i>Cooperia oncophora</i>	9B	Ruminant	ESP from adult	AB 4800 MALDI-TOF/TOF MS		[84]
<i>Haemonchus contortus</i>	9B	Ruminant	ivermectin-susceptible & -resistant adult female	W MALDI-TOF MS		[88]
			adult tissue	BD amaZon ETD MS		[89]
			sheathed L3, exsheathed L3	ABI Sciex 5800 MALDI-TOF/TOF MS		[90]
<i>Heligmosomoides polygyrus (bakert)</i>	9B	Mouse	ESP from adult	BD maXis UHR-TOF MS		[101]
	9B	Mouse	ESP from adult	W QTOF micro MS		[100]
<i>Necator americanus</i>	9B	Human	L3, adult	AB Sciex 5600 MS/MS		[79]
<i>Nippostrongylus brasiliensis</i>	9B	Rat	ESP from L3 and adult	ABI Sciex Triple TOF 5600/5800 MS		[103]
<i>Oesophagostomum dentatum</i>	9B	Swine	drug-treated L3	BD Ultraflex II MALDI-TOF/TOF MS		[96]
			sheathed L3, exsheathed L3	BD Ultraflex II MALDI-TOF/TOF MS		[97]
<i>Teladorsagia (Ostertagia) circumcincta</i>	9B	Sheep, Ruminant	ESP from infective L3	BD Esquire HCTplus ESI MS		[92]
			ESP from L4	TF Electron LTQ ion trap MS		[93]
			extracellular vesicles in ESP from L4	BD Esquire HCTplus ESI MS		[94]

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Abbreviations: Applied Biosystems (AB); Bruker Daltonics (BD); European Bioinformatics Institute (EBI); EBI European Nucleotide Archive (ENA); excretory/secretory products (ESP); mass spectrometer (MS); matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF); National Center for Biotechnology Information (NCBI); NCBI BioProject (BP); NCBI Gene Expression Omnibus (GEO); NCBI Sequence Read Archive (SRA); Thermo-Fisher/ThermoFisher Scientific (TF/TFS); Waters (W); Xiamen University, China (XU)