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Lessons from the genomes and transcriptomes of filarial nematodes

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Abstract

Human filarial infections are a leading cause of morbidity in the developing world. While a small arsenal of drugs exists to treat these infections, there remains a tremendous need for the development of additional interventions. Recent genome sequences and transcriptome analyses of filarial nematodes have provided novel biological insight and allowed for the prediction of novel drug targets as well as potential vaccine candidates. In this review, we discuss the currently available data, insights gained into the metabolism of these organisms, and how the filaria field can move forward by leveraging these data.

Keywords

filaria; parasitic nematodes; genomics; transcriptomics

Introduction

Nematodes cause the most common parasitic infections in humans, and the tissue-dwelling filarial worms produce the most severe pathology associated with these infections. Current control programs, universally based upon the mass distribution of a small arsenal of drugs targeting microfilaria loads and transmission, will not be sufficient on their own to eliminate lymphatic filariae by 2020, especially where loasis is co-endemic. The programs are also threatened by the difficulties of repeatedly achieving high coverage rates necessary for elimination when using the currently available drugs, as well as by the specter of drug resistance to the limited selection of drugs now being used. No method to permanently sterilize the adult female worms or to kill the filarial parasite exists, sounding the call for additional research to discover novel macrofilaricidal drug candidates. Many filarial worm species carry a *Wolbachia* endosymbiont that can be eliminated through antibiotic treatments, interfering with development, reproduction, and survival of the worms within the host, a clear indicator that the *Wolbachia* are crucial for survival of the parasite. Recent

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advances in the sequencing of filarial genomes and transcriptomes, as well as those of their bacterial endosymbionts, have contributed greatly to our better understanding of the biology of these parasites. They have also enabled the identification of novel drug targets and potential vaccine candidates.

Current state of genome and transcriptome sequencing efforts

Filarial nematodes currently infect up to 54 million people worldwide, with many millions more at risk for infection, representing the leading cause of morbidity in the developing world [1, 2]. Lymphatic filariasis, due to *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*, represents the leading cause of morbidity. It is followed by onchocerciasis and loasis, caused respectively by *Onchocerca volvulus* and *Loa loa*. The study of these important pathogens is made particularly difficult due to the inability to maintain most of these parasites outside of the human host, requiring extraction from human blood or tissue. *B. malayi* remains the only human filarial parasite that can be maintained in small laboratory animals [3–6]. Thus, the generation of high- quality genomes and transcriptomes is essential to interpret critical proteomic information, and to study the unique biology of these different filarial parasites. To this end, sequencing efforts have led to the first high-quality genome assemblies with reconstructions of whole chromosomes for both *O. volvulus* [7] and *B. malayi* (WormBase.org) [Manuscript in preparation]. A number of draft genomes also exist for the other filarial nematodes including *L. loa*, *W. bancrofti*, *B. pahangi*, *D. immitis*, and *O. ochengi* [8–10] [Figure 1].

Genome Features

Filarial gene content

The evolution of a parasitic lifestyle is associated with a reduction in genome size, reflective of the parasite's dependency on its host [11]. Sequenced genomes of filarial nematodes such as *B. malayi* and *O. volvulus* encode 13,865 and 12,143 genes, respectively, a little more than half the number of protein coding genes present in the *C. elegans* genome [7, 12]. Meanwhile, the genomes of *L. loa* and *W. bancrofti* are predicted to encode a slightly larger number, at 14,261 and 14,496-15,075, respectively [8]. While this means we can expect to find a number of reduced protein families, we would also expect to see expansion of gene families involved in the adaptation to parasitism. For example, as compared to the other filariae, the *L. loa* genome was found enriched for a number of protein domains including the pyridoxamine 5'-phosphate oxidases that synthesize vitamin B, as well as for chemoreceptors, potentially indicative of complex interactions between *L. loa* and its environment [8]. *W. bancrofti* was found to be enriched for domains related to cellular adhesion and the extracellular matrix, potentially involved in either mediating fibrosis associated with lymphatic filariasis, or establishment of infection in the lymphatics [8]. In *O. volvulus* using a comparative analysis approach it was found that the gene family encoding the alpha subunit of the enzyme collagen prolyl 4- hydroxylase (C-4PH alpha), an enzyme involved in collagen synthesis, was significantly expanded as compared to other filariae [7]. Similarly, G-protein-coupled receptors (GPCRs) show a remarkable expansion in *O. volvulus*, with the Srx and Srsx GPCR families among the most duplicated gene families in the genome [7]. Conversely, the nuclear hormone receptor (NHR) gene family was found to

be particularly depleted in filarial worms as compared to free living nematodes like the *Caenorhabditis*—*B. malayi* has 50 and *O. volvulus* has 24 classical NHRs and 8 orphan receptors, as compared to *C. elegans* which has over 270 NHRs. Interestingly, the *O. volvulus* ecdysone receptor (OVOC9104) was also gained at the base of the filarial lineage. Ecdysone and related hormones regulate molting and metamorphosis in arthropods, leading to speculation that this gain in NHRs may be an adaptation to the insect host [7].

Gene synteny among nematode genomes

Comparisons of the genome organization of the free living nematode *C. elegans* to that of parasitic nematodes reveal that while local synteny—or conservation of gene order—has been lost, linkage has been conserved on chromosomal units [7, 12, 13]. Among the filariae there is a relatively high level of conservation of gene order, with 40% of *L. loa* genes being syntenic with *B. malayi* genes and 13% relative to *W. bancrofti* [8]. However, because nearly all syntenic breaks occurred at scaffold ends, it is likely that the true level of synteny is actually much higher [8]. A study to analyze macro- and microsynteny across nematodes found that intra- rather than inter-chromosomal rearrangements were favored [13]. The relative syntenic conservation values were also shown to be consistent with the estimated evolutionary distance to *C. elegans*. For example, the syntenic conservation score is 0.752 between *C. elegans* and *C. briggsae*, and 0.508 between *C. elegans* and *B. malayi*. Whole genome analysis of *B. malayi* and *B. pahangi* revealed high synteny, with a genome coverage range of 70-75%, with 90% of predicted genes in *B. pahangi* containing orthologs in *B. malayi* [9]. The existence of syntenic blocks reveals a likely functional constraint on genome evolution [13].

The evolution of sex chromosomes

One unique observation from these sequenced genomes and the assembly of full filaria chromosomes is the presence of a Y chromosome in both *B. malayi* and *O. volvulus*, unlike the model nematode *C. elegans* which has an XX/X0 sex determinism [14]. Evidence for a Y chromosome in these organisms has existed for almost 30 years, however, until recently, we had been unable to determine its sequence, gene content and origin [15, 16]. It was hypothesized that the nematode ancestor had a Y chromosome that was eventually degraded and lost in *C. elegans*, requiring sex-determining genes to jump to autosomes. The absence of a Y chromosome in most other nematodes suggests the X0 sex chromosome system is the ancestral state, and the XY system is a derived, neo-XY system that evolved from the fusion of the X chromosome and an autosome [17, 18]. Currently, Y chromosomes have also been observed in 10 other filarial nematodes besides *B. malayi* and *O. volvulus*: *Dirofilaria immitis*, *Brugia pahangi*, *Wuchereria bancrofti*, *Onchocerca gutturosa*, *Onchocerca lienalis*, *Onchocerca tarsicola*, *Onchocerca armilatta*, *Onchocerca dukei*, *Onchocerca ochengi*, and *Onchocerca gibsoni* [15, 16, 19–23]. However, these observations were made through karyotyping; *B. malayi* and *O. volvulus* are the only genomes of sufficient quality to observe the sequence of the Y chromosome. In nematodes where the Y chromosome determines sex, it is hypothesized that the selection against chromosomal rearrangements in the X chromosome would be relaxed.

***Wolbachia*, the bacterial endosymbiont**

Wolbachia genomes and lateral gene transfer

No discussion of filarial genomes and transcriptomes is complete without a mention of *Wolbachia*, the bacterial endosymbiont of most filaria. *Wolbachia* are intracellular bacteria found in both arthropods such as insects, mites, and crustaceans, as well as filarial nematodes. There is currently only one species in the genus *Wolbachia*, *Wolbachia pipientis*, divided into six supergroups, A-F. *Wolbachia* found in filarial nematodes belong to supergroups C and D [24]. The first filarial *Wolbachia* genome to be sequenced was that of the *Wolbachia* endosymbiont of *B. malayi* (*wBm*) of supergroup D, which was found to be 1.1 Mb with 805 protein coding genes [25], smaller than previously described *Wolbachia* genomes. Interestingly, the genome maintains complete pathways for the synthesis of heme, riboflavin, flavin adenine dinucleotide (FAD), as well as pathways for *de novo* synthesis of purines and pyrimidines. This observation led to the speculation that because the *B. malayi* genome is lacking these complete synthesis pathways, *wBm* may be provisioning the nematode with these essential cofactors [12, 25]. Sequencing of the *Wolbachia* genome of *O. ochengi* (*wOo*) of supergroup C revealed a genome 11% smaller than that of *wBm*, with several regions of microsynteny with *wBm*, but generally large differences in chromosomal organization [26]. The *wOo* genome is the most reduced of all *Wolbachia* genomes sequenced to date, having lost features such as transposable elements; the genome contains only six insertion sequence (IS) copies, and has no group II introns, unlike the *wBm* genome and the *Wolbachia* of insects where they are found in high abundance [25]. The recent sequencing of the *Wolbachia* endosymbiont of *O. volvulus* (*wOv*) also of supergroup C, revealed a genome with 785 predicted protein-coding genes, nearly identical to *wOo*, but about 1,937-bp larger [7]. A large number of SNPs (4,400) were also identified, of which 57 may result in functional differences, potentially involved in the interaction of the endosymbionts with their filarial and/or mammalian hosts (i.e. cattle and humans) [7].

Nuclear *Wolbachia* transfers (*nuwts*) have been identified in all *Wolbachia*-colonized and *Wolbachia*-free filarial genomes sequenced thus far, suggesting that all filarial nematodes were once colonized by *Wolbachia*, with some having since lost the endosymbiont [8, 27]. The size and predicted functionality of these transfers are quite variable between filarial nematodes. While *L. loa* (a *Wolbachia*-free filaria) contains some presumably old *nuwts*, none are over 500 bp and there is no evidence that any of them are expressed and functional [8]. Conversely, *nuwts* are prevalent in two of the other *Wolbachia*-free filarial nematodes, *Acanthocheilonema viteae* and *O. flexuosa*, with average sizes of 173.6 and 158.9 bp respectively [27]. Of the 49 *Wolbachia*-like DNA sequences found in the *A. viteae* genome, 30 were detected by qRT-PCR to be expressed; of the 114 *Wolbachia*-like sequences found in the *O. flexuosa* genome, 56 were expressed [27]. There is also evidence of horizontal gene transfer in the genomes of filaria that have maintained *Wolbachia* infection, such as *B. malayi*. The *B. malayi* genome contains 227 *Wolbachia* genes and gene fragments, 32 of which contain open reading frames and thus potentially encode functional proteins. Evidence for the expression of 4 of the transfers was found in the *B. malayi* transcriptome [28]. The *O. volvulus* genome contains 531 putative *nuwts*, all but 4 of which were fragmented. While these 4 transfers were confirmed by amplification, cloning, and sequence

verification of the *Wolbachia*-nematode junction, the nuwts in *O. volvulus* appear to be largely nonfunctional [7].

Concurrent transcriptional analysis

In addition to genome analyses, next generation sequencing has enabled the concurrent stage-specific transcriptional profiling of both the filarial nematode and the bacterial endosymbiont. Such studies have been completed in *D. immitis*, *O. ochengi*, and *B. malayi* [26, 29, 30] [Manuscript under review at PLoS NTD]. In all studies, the transcripts for the chaperonin and heat shock proteins including GroEL, GroES, and DnaK were among the most highly expressed. All three studies point to the potential stage-specific provisioning of metabolic products by *Wolbachia* to the filarial worms as well as correlated pathway expression. A better understanding of the specific mechanisms that define the mutualistic interplay between the filariae and their *Wolbachia* endosymbiont will facilitate the identification of pathways that could be targeted to ultimately kill the adult worms; such novel macrofilaricidal drugs would complement present MDA efforts to control and eventually eliminate filariasis.

Establishment of infection in the final host

Tissue migration strategies

Serine and cysteine proteases have been identified as integral to host invasion and migration in a number of parasitic nematodes. *Trichuris muris*—a parasitic nematode of mice—uses serine proteases to break down the mucus barrier of the host intestine to establish infection [31]. Cercarial elastases and trypsin-like serine proteases in *Schistosoma mansoni* have been shown to play a critical role in tissue invasion of the human host as well as egression from the intermediate snail host, while *S. japonicum* and *Trichobilharzia regenti* appear to use the Cathepsin B peptidase for their tissue invasion strategy [31–34]. Analysis of the newly sequenced *O. volvulus* genome reveals that while there are no cercarial elastase-like enzymes present, there are 8 trypsin-like serine proteases as well as one chymotrypsin-like protease in the genome [7]. Genome-wide analysis of filarial genomes shows that there are four duplications of trypsin-like serine proteases in the filarial nematodes. Analysis of their expression reveals stage-specific regulation wherein the chymotrypsin-like protease is expressed across the larval stages as well as in the microfilaria, while six of the trypsin-like serine proteases show significant upregulation in the adult males. This pattern of expression suggests the importance of trypsin-like proteases in certain stages, such as for *O. volvulus* adult males that must migrate through subcutaneous tissue of the host to fertilize females that reside in nodules.

Molting

The L3 to L4 molt in filarial parasites occurs immediately upon infection of the mammalian host marking the establishment of infection. Both the *B. malayi* and *O. volvulus* genomes contain homologs to most of the genes required for molting in *C. elegans*, including proteases, NHRs, cuticular collagens, and chitinases [12, 35]. As previously mentioned, analysis of NHRs in the *O. volvulus* genome revealed significantly fewer NHRs than in *C. elegans*. Despite this, *O. volvulus* has maintained orthologs to all 5 *C. elegans* NHRs

exclusively involved in molting and metamorphosis [7]. Unlike *C. elegans*, the L3 to L4 molt in several filarial nematodes has additionally been shown to be dependent on the activity of CPL-1 and CPZ-1, members of the cathepsin L-like and cathepsin Z-like filarial proteases [36, 37]. Analysis of CPL gene families revealed an expansion of CPL-like enzymes in the filarial clade, and an additional expansion in the *O. volvulus* genome. Transcriptome analysis in *O. volvulus* also revealed significant regulation of CPL and CPZ molecules in the L2 and L3 larvae as compared to other stages [35]. Their increased expression in L2 larvae suggests that CPLs and CPZ also play a role in the L2 to L3 molt in the vector, similar to what was described in *B. malayi* [38].

Signaling

Another component of niche specificity has to do with how parasitic nematodes receive signals from their environments. To begin to understand this process, a genome-wide analysis of GPCRs in free-living and parasitic nematodes was performed [8]. It was found that filarial genomes were lacking a wide array of GPCRs as compared to free-living *C. elegans*—only the *srab*, *srx*, *srsx*, and *srw* families were conserved across all nematodes, suggesting that these GPCRs mediate vital nematode functions [8]. In common with other filaria, *O. volvulus* is missing three of the families present in *C. elegans* and *C. briggsae*, including *Srj*, *Sra*, and *Srb*. However, unlike other filaria, *O. volvulus* contains members of all other GPCR families represented in *C. elegans* and *C. briggsae*. We also find that the *str* family of GPCRs, hypothesized to be involved in the detection of volatiles or odorants, is lacking in all the filaria and other nematodes that live in only aqueous environments [8]. The maintenance of *str* and other families of GPCRs in *O. volvulus* that are lacking in other filariae suggests their importance in the *O. volvulus* lifecycle [7].

Insights into host-parasite interactions

Immunomodulation

Filarial parasites are natural immunomodulators, able to subvert the host immune responses in order to successfully persist in both their human and insect hosts. It is hypothesized that filaria use not only their own innate immune system, but are able to manipulate that of their host [8]. While the exact mechanisms of such manipulation remain largely unknown, genomic analysis has shed light on these processes. A comparative analysis of filarial genomes revealed the presence and expansion of a number of gene families implicated in immunomodulation in *L. loa* [8]. While adaptive immune molecules such as immunoglobulins and Toll-like receptors are absent in filarial genomes, there is evidence for a primitive Toll-related pathway in many filaria [7, 8]. Other members of the innate immune system of *L. loa*, *B. malayi*, and *O. volvulus* include lectins, galectins, jacalins, as well as scavenger receptors [7, 8, 12]. A number of serpins, small serine protease inhibitors (SPIs), and cystatins that are known to interfere with antigen presentation and processing in T cells, were also found in the genomes of *L. loa*, *O. volvulus*, *B. malayi*, and *D. immitis* [7, 8, 10, 12, 39]. SPIs also play an important role in controlling the molting process [40] and immune evasion [41]. Notably, the analysis of the *O. volvulus* genome revealed the presence of 10 additional SPIs beyond the 2 previously identified, Ov-SPI-1 and Ov-SPI-2 [35]. Other potential immune modulators including members of the macrophage migration inhibition

(MIF) family and transforming growth factor beta (TGF β) homologs have been identified in *B. malayi*, *O. volvulus*, *L. loa*, and *D. immitis* [7, 8, 10, 12]. In addition, a variety of human cytokine and chemokine mimics or antagonists were identified in filarial genomes [7, 8, 10, 12]. So far, no filarial genomes have been found to encode the antibacterial peptidases seen in *C. elegans* and other non-filarial nematodes [7, 8, 12].

Kinases

Protein kinases, particularly those that are unique to nematodes and distinct from the ones in humans, represent attractive drug targets and their analysis can provide biological insight. A comparison of protein kinases in filarial and non-parasitic nematodes revealed many differences, particularly regarding those involved in meiosis. For example, the genome of *L. loa* contains the widely conserved TTK kinase (MSP1) involved in eukaryotic meiosis, which is absent in *C. elegans*. Conversely, filarial nematodes lack the highly conserved CHK-2 kinase, of the RAD53 family found in *C. elegans* [8]. The *B. malayi* genome was found to have 205 conventional and 10 atypical protein kinases, of which 142 are essential, based on RNAi evidence in *C. elegans* [12]. Analysis of protein kinases in *O. volvulus* revealed 168 kinases without significant human matches [7]. Analysis of the *L. loa* genome revealed six orthologs to targets of drugs already approved for use in humans, including the tyrosine kinase inhibitor imatinib, effective against schistosomes [8]. Initial studies have already shown the distinctive effects of the tyrosine kinase inhibitors imatinib, nilotinib, and dasatinib on *B. malayi* at different stages in the life cycle, including adult males, adult females, L3 larvae, and microfilariae *in vitro* [42].

Where do we go from here?

Diseases caused by parasitic nematodes constitute the leading cause of morbidity in the developing world. Current microfilaricidal drugs appear to be insufficient for the ultimate goal of control and elimination of these parasitic infections, necessitating a better understanding of the basic biology of these worms and the development of new drugs and vaccines. With the nearly complete filarial genomes and the availability of stage-specific transcriptomic and proteomic data, there now exists a plethora of information and new tools with which to achieve these goals. By using the newly available genomic and transcriptomic data significant progress has already been made towards the identification of novel vaccine and drug targets. Some of these proteins are already the targets of already approved human drugs that have not yet been tested on the filariae but that could potentially be used as new therapies for filarial infections. A computational target-based approach to screen FDA-approved drugs across all World Health Organization Anatomical Therapeutic Classes (WHO ATC) with the potential for use as anthelmintics has led to the identification of 16 *O. volvulus* proteins likely to be good drug targets; they are mostly enzymes and proteins involved in ion transport and neurotransmission [7].

Identification of novel drug targets

Metabolic potential has been shown to be a critical determinant of a pathogen's ability to survive in an infected host [43–45]. Genome-scale metabolic reconstruction is a way to determine an organism's metabolic potential as well as allow for *in silico* investigation of

essential pathways. Flux balance analysis (FBA) is increasingly being used to create draft metabolic networks based on available enzyme annotation data to better understand the metabolic potential of a pathogen [46]. FBA enables the identification of metabolic fluxes that maximize certain functions such as growth potential, using a biomass equation describing the proportion of metabolites required by a system for growth. Metabolic reconstruction has been used to predict enzymes required for growth and virulence of a number of pathogens including *Plasmodium falciparum* [47], *Leishmania major* [48], and *Toxoplasma gondii* [44]. FBA was used for the first time to reconstruct the metabolism of filarial nematodes, comparing the essential reactions in *L. loa* and *O. volvulus* and the contribution of *Wolbachia*. For *O. volvulus*, only 71 reactions were predicted to be essential, while for *L. loa*, 112 reactions were predicted to be essential. *O. volvulus* benefits from *Wolbachia* contributions to fatty acid metabolism, heme synthesis, and purine and pyrimidine metabolism, which, unlike *L. loa*, does not require the salvage of precursors or biomass components involved in these pathways. Analysis of purine metabolism reveals that *Wolbachia* provides an alternate pathway for purine metabolism, whereas *L. loa* depends exclusively on adenine import. This pathway is an example of how the presence of *Wolbachia* can change the metabolic chokepoints in filaria and serves to identify selective drug targets [7].

Identification of novel vaccine candidates using immunomics

Newly available genomic and proteomic data for parasitic nematodes is making high-throughput vaccine antigen discovery, known as “immunomics”, now possible [49–52]. This approach allows for profiling of host immune antibody response to candidate parasitic antigens in a high throughput manner. This assay was employed to identify potential vaccine antigens in the genomes of *S. mansoni* [52, 53], *S. Japonicum* [50], *Necator americanus* [51], and for the first time also in the genome of *O. volvulus* [35]. Criteria for selecting *O. volvulus* candidate vaccine proteins on the array have included: 1) abundant expression in L3 and molting L3 or in microfilariae, the targets for prophylactic and therapeutic vaccines, respectively [54], 2) predicted secretion based on the presence of a leader sequence or other motifs, containing 0-3 transmembrane domains and below 50 kDa in size (to increase the probability of efficient expression using the cell-free *in vitro* transcription and translation systems), and 4) not having a significant human match. These selection criteria focused on proteins that are likely to be on the apical surface or secreted by targeted parasites, and are therefore more likely to come into contact with the host immune system [55]. There is evidence that natural immunity against certain parasitic nematodes, including *O. volvulus*, can be acquired in a few individuals in affected populations; these individuals are known as putatively immune [56]. It has been shown that this immunity is facilitated by a protective immune response against the infective stage (L3) larvae, suggesting that excretory-secretory products and surface proteins of L3 larvae are an important source of protective antigens [56]. The array was screened with sera samples from putatively immune individuals, infected individuals, and control individuals (individuals from non- endemic areas). The IgG1, IgG3 and IgE antibody/antigen recognition profiles were compared to naturally immune individuals and those infected. Elevated cytophilic IgG1 and/or IgG3 responses to L3 antigens were previously shown to be associated with protection [57]. Although IgE antibodies could also be protective [58], we tested IgE responses to reduce the risk that

administration of such vaccine candidates in humans could generate atopic responses, an essential requirement for helminth-derived recombinant vaccine antigens [59]. A recent immunomic analysis in *O. volvulus* led to the identification of six novel vaccine candidates based on IgG1 and/or IgG3 reactivity, with little to no IgE reactivity [35] [Table 1]. Notably, the majority of these candidates are hypothetical, with no known function.

Conclusion














The abundance of genomic, transcriptomic, and proteomic data has provided novel biological insight into filarial nematodes, as well as allowed for the identification of novel drug targets and vaccine candidates. However, most of these analyses rely on annotated genes with orthologs in other species, such as *C. elegans*. The next challenge will be to assign functions to the many genes annotated as “uncharacterized” or “hypothetical proteins” in both the filarial and *Wolbachia* genomes through the development of high-throughput methods. It is only with the understanding of these hypotheticals that we will be able to go even further in exploring the novel biology of filarial parasites and their bacterial endosymbionts.

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Organism	Host	Date	Data Available			Wolbachia Data Available		
			 Genome	 Transcriptome	 Proteome	 Genome	 Transcriptome	 Proteome
<i>L. loa</i>		2013 ¹	✓	Mf ¹		NA		
<i>W. bancrofti</i>		2013 ¹	✓					
<i>O. volvulus</i>		2016 ²	✓	L2, L3, L3D1, L3D2, L3D3, L4 AF, AM, Mf ⁶	L3, L3D1, L3D2, L3D3, L4, AF, AM, Emb ⁶	✓		
<i>B. malayi</i>		2007 ³	✓	L3 ⁷ , L4 ^{7,8} , F30 ⁸ , F42 ⁸ , AF ^{7,8} , M30 ⁸ , M42 ⁸ , AM ^{7,8} , Mf ⁷	L3, AF, AM, Mf ¹¹	✓	L4, F30, F42, AF, M30, M42, AM ⁸	L3, AF, AM, Mf ¹¹
<i>B. pahangi</i>		2015 ⁴	✓	Mf ⁹				
<i>D. immitis</i>		2012 ⁵	✓	L3, L4, AF, AM, Mf ¹⁰	AF, AM ¹²	✓	L3, L4, AF, AM, Mf ¹⁰	
<i>O. ochengi</i>		2016 ²	✓		L3, AF, AM, Mf ¹³	✓	AF, AM ¹⁴	AF ¹⁴

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Figure 1. Summary of Omics data available for filarial parasites

The genomic, transcriptomic, and proteomic data available for filarial nematodes with sequenced genomes. Stages for which transcriptomic and proteomic data exist are listed in each box, L2, L3, L4: larval stages, L3D1: L3 day 1 of molting, L3D2: L3 day 2 of molting, L3D3: L3 day 3 of molting. F30: juvenile female 30 days post infection (dpi), F42: juvenile female 42 dpi, M30: juvenile male 30 dpi, M42: juvenile male 30 dpi, AF: adult female, AM: adult male, Mf: microfilaria, Emb: embryo.

Table 1**Six novel vaccine candidates for *O. volvulus***

Table of the six novel vaccine candidates identified by the immunomic analysis in *O. volvulus*. Expression values in Reads Per Kilobase of transcript per Million mapped reads (RPKM) are shown for each developmental stage.

Gene	Description	Expression (RPKM)							
		Skin Mf	Nodular Mf	L3	L3 Day1	L3 Day3	Adult Male	Adult Female	
OVOC8619	Adhesion regulating molecule conserved region family protein	119.1	141.9	26.8	116.9	124.5	99.3	41.0	
OVOC7083	hypothetical protein Bm1_50435	27.4	257.9	72.9	29.6	94.8	63.1	67.6	
OVOC11598	hypothetical protein LOAG_10445	2.2	66.7	58.8	9.7	9879.0	1.2	23.4	
OVOC10819	hypothetical secreted protein precursor	0.2	2.3	0.5	1.6	1.9	153.3	1.0	
OVOC5395	hypothetical protein Bm1_06245	23.9	101.9	0.6	5.4	38.7	13.1	14.7	
OVOC12235	hypothetical protein LOAG_18989	0.5	0.5	336.4	31.7	235.9	2.1	2.0	