A Large-scale Huntingtin Protein Interaction Network Implicates Rho GTPase Signaling Pathways in Huntington’s disease

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Background: Huntington’s disease is a fatal neuropsychiatric disorder caused by aberrant protein folding and interactions.

Results: An interaction network comprised of primary and secondary huntingtin interacting proteins is significantly enriched for pathways implicated in HD including RhoGTPases.

Conclusions: Huntington interacts with members of the Rho GTPase signaling pathways and regulates filapodial dynamics.

Significance: This protein interaction network provides a resource for HD target discovery.

ABSTRACT

Huntington’s disease (HD) is an inherited neurodegenerative disease caused by a CAG expansion in the HTT gene. Using yeast two-hybrid methods, we identified a large set of proteins that interact with huntingtin (HTT) interacting proteins. This network, comprised of HTT interacting proteins (HIPs) and proteins interacting with these primary nodes, contains 3,235 interactions among 2,141 highly interconnected proteins. Analysis of functional annotations of these proteins indicates that primary and secondary HIPs are enriched in pathways implicated in HD, including mTOR, Rho GTPase signaling and oxidative stress response. To
validate roles for HIPs in mutant HTT toxicity, we show that the Rho GTPase signaling components, BAIAP2, EZR, PIK3R1, PAK2 and RAC1 are modifiers of mutant HTT toxicity. We also demonstrate that Htt co-localizes with BAIAP2 in filopodia and that mutant HTT interferes with filopodial dynamics. These data indicate that HTT is involved directly in membrane dynamics, cell attachment and motility. Furthermore, they implicate dysregulation in these pathways as pathological mechanisms in HD.

INTRODUCTION

Huntington’s disease is an autosomal dominant neurodegenerative disease caused by a CAG repeat expansion in the first exon of the gene encoding the protein huntingtin (HTT) (1). HD manifests with progressive motor and psychiatric impairments caused by neuronal dysfunction and loss in the cortex and striatum (2,3). Huntingtin is involved in a variety of cellular functions including vesicle transport, transcription, and energy metabolism (4). HD pathogenesis is generally thought to result from a combination of a gain of toxic properties by mutant HTT as well as a loss of normal huntingtin function (5). Huntingtin is a ~350 kDa protein containing a polyglutamine (polyQ) region, a PRR (proline-rich region), HEAT (Huntingtin, elongation factor 3, protein phosphatase 2A, target of rapamycin 1) repeats, and a number of caspase cleavage sites (4,6). Several studies have emphasized a critical role of misfolded N-terminal fragments of mutant HTT (7,8) that are natural products of HTT processing (9). HTT is known to have a large number of interacting proteins involved in a diverse range of biological processes. Numerous studies have shown that polyQ expansion in HTT may alter biological processes that are essential to cellular homeostasis and neuronal survival through impairment of its protein binding activities (10-13).

A number of large-scale screens aimed to elucidate new pathways involved in HD pathogenesis by defining HTT partners using yeast-two-hybrid (Y2H) and affinity purification approaches (14-17). Analyses of binary interactions or complexes identify the first level of HTT interacting proteins. We have previously described a protein interaction network derived from a comprehensive Y2H screen using HTT as a bait. That study reported 102 high-confidence HTT interacting proteins and many of these were shown to be modifiers of mutant HTT toxicity in a Drosophila model of HD (16). In this study, we report Y2H screening results for these primary HTT interacting partners derived from a genome-scale interaction map (18,19). Using the 102 HTT primary partners identified in our first screen, we identified a secondary interactome of 2038 known partners to build an expanded huntingtin protein interaction network (HDNet). This network includes HTT-primary, primary-primary, and primary-secondary interacting proteins. We analyzed the connectivity properties of these proteins at the two levels, showing significantly high interconnectivity between HDNet members compared to random proteins in a global curated interaction network from HPRD (Human Protein Resource Database).

Integration of data from different ‘omics’ approaches has been shown to improve functional annotations and to help to formulate biological hypotheses (20). Combination of biological annotations with integration of gene expression data from post-mortem HD brain highlighted the role Rho family GTPase signalling proteins in HD pathology. Using cells models, we showed that components of this pathway are modifiers of expanded polyQ-induced toxicity. We also showed that mutant HTT interferes with BAIAP2-induced filopodia formation, validating the role of Rho signalling in mutant HTT toxicity. Our study provides a comprehensive resource of binary protein interactions that define novel pathways contributing to Huntington’s disease pathology.

EXPERIMENTAL PROCEDURES

Y2H screen - Y2H screens were performed as previously described (21). Screen for primary HTT partners has been described in Kaltenbach et al. (16). Interactions of primary partners have been extracted from a genome-wide Y2H screen as described previously (18). Y2H screens were performed in 96-well plates by mating in each well 5x10⁶ cells of a yeast clone expressing a single bait with 5x10⁶ clonally diverse cells from a prey library. After mating overnight, the matings were plated onto medium that selected simultaneously for the mating event, the expression of the ORF-
selection markers, and the activity of the metabolic reporter genes, ADE2 and HIS3. Yeast that grew on this selection medium (‘‘positives’’) were counted and transferred into liquid medium in a 96-well format. Cloned inserts were amplified from plasmid PCR. Liquid cultures grown from positive yeast colonies were used as templates in PCR reactions that amplified either both bait and prey cDNA inserts, or prey inserts only in screens in which the baits had been sequenced before the matings.

To determine identity of the coding genes from which the primary and secondary sequence fragments were derived, we first aligned cDNA fragments to the set of human RNA sequences downloaded from NCBI (ftp://ftp.ncbi.nih.gov/refseq/H_sapiens/mRNA_Prot/human.rna.gpff). All Y2H search data and DNA sequences used to determine interaction pairs reported in this study are included in Table S2.

Network topology analysis - HTT primary and secondary proteins were anchored to the PPI network defined by HPRD (release 9). The shortest distances between any two nodes in the PPI network were computed through the Matlab package MatlabBGL. The largest subgraph contains 9,219 proteins, and 64 primary and 1,167 secondary proteins could be found in this subgraph, respectively. All network metrics were based on this largest subgraph. To approximate the distribution of the shortest distance observed in HPRD, we randomly sampled 1 million networks with each having 64 nodes as controls to the primary proteins, and 100,000 networks with each having 1,167 nodes as controls to the secondary proteins.

Functional annotation of the network - Total data set and the subgroups of primary and secondary partners of HTT were analyzed using Ingenuity Pathway Knowledge Base (Ingenuity System, Mountain View, CA). The three groups were subjected to IPA Core analysis. Canonical Pathways were considered as significantly enriched with a FDR corrected p-value<0.01. Enriched canonical pathways were ranked based on corrected p-value obtained for the whole HDNet dataset, and represented using IPA comparison analysis tool. The most significantly enriched lists were visualized in Cytoscape (22), using a union of HDNet and HPRD networks (23). Proteins were represented using node color for HD dysregulation data (24,25) based on significance level (corrected p-value<0.01) and node shape for level of interaction with HTT (primary/secondary). Edges were represented as lines and dashed lines based on the source of interaction, HDNet and HPRD respectively. Only connected nodes were represented in figure 4 and supplemental figure 2 for better visibility, information of all nodes is found in Table S4.

Domain identification - The mapped protein segments were searched against NCBI CDD (version 3.05) to obtain domain information, with settings of E-value cut-off 0.01 and filtering low-complexity regions. Whenever multiple domains were found on the same protein segments, only the most specific hits were kept. By definitions from CDD, the specificity of hits decreases in the order of specific domain, superfamily, and multiple domains.

Plasmids, siRNA and lentiviruses - BAIAP2 cDNA was obtained from Open Biosystems (MHS1011-59012), PCR-amplified with Pfu Turbo (Agilent) as recommended by manufacturer, with the following primers: forward primer, 5’-CACCATGGCTCTGTCTCGCTC-3’; reverse primer, 5’-TGGCCATCTGCTGAGGAG-3’. cDNA was next cloned into the Gateway pENTR-D-TOPO vector; C-terminal V5-tagged BAIAP2 was generated by LR reaction with pDEST40 following the manufacturer’s protocol (Invitrogen). Clones were verified with sequencing at all the steps. pDEST40-LacZ was used as a negative control. Entry clones containing HTT N-terminal fragments 1-558 (with 23Q or 135Q) fused to a C-terminal GFP tag have been described in (26). LR reaction was performed with pLenti CMV Puro DEST vector (w118-1, Addgene); integrity of clones and length of CAG repeats were verified by sequencing at all steps. Lentiviral production and infection were performed in HEK293FT as described in (27). Full-length HTT plasmids were described in (28). Dharmacon siGENOME SMARTpools (Thermo Scientific) were used for siRNA disruption of CASP3 (M-043042), BAIAP2 (M-046696), EZR (M-046568), PI3KR1 (M-041079), PIK3R2 (M-041085), PIK3R3 (M-041300), PAK2 (M-040615), WAVE
Cell culture and transfection - Mouse striatal cell lines expressing full-length HTT with either wild-type (7Q/7Q) or mutant (111Q/111Q) polyglutamines were handled as previously described (29), maintained at 33°C in a humidified atmosphere of 95% air and 5% CO₂ in DMEM containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were nucleofected using kit L (Amaxa) with 3 μg of siRNA for two millions of cells, as previously described (28). Mouse NIH-3T3 and human HEK293T cells were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO₂ in DMEM containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Infection of HEK293T cells was performed as described in (27). Cells were selected with puromycin (Invitrogen) at the concentration of 1 μg/ml of culture media 48 hours after infection. NIH-3T3 and HEK293T cells were transfected using lipofectamine 2000 reagent following manufacturer’s protocol (Invitrogen), with full-length HTT-17Q, -138Q, C-terminal V5-tagged BAIAP2 or Dharmacon smart pool BAIAP2 siRNA.

Caspase assays - Cell death assays were performed as previously described (28). Briefly, transfected cells were plated on collagen-treated 96-well plates and incubated for 48 hours. After 24 hours of serum starvation, activation of caspase 3/7 was measured in cells using Apo 3/7 HTS High Throughput Screen Assay kit (Cell Technology), using Fusion Alpha-FP HT (Perkin Elmer) microplate reader with excitation and emission wavelengths of 420 and 520 nm, respectively. Toxicity is represented as caspase 3/7 activity in dRFU/min/mg of protein, relative to STdh<sup>111Q/111Q</sup> treated with non-targeting siRNA pool. Tests were performed in triplicates; t-test was used for statistical analysis (GraphPad prism).

Western blotting and co-immunoprecipitation - Cells were seeded on 6-well plates, harvested and lysed with M-PER (Thermo scientific) 72 hours after transfection for western blotting. Primary antibodies anti-BAIAP2, PIK3R1 (Genscript), Ezrin, PAK2 (Cell Signaling), PIK3R2 and PIK3R3 (R&D Systems), RAC1 (Sigma-Aldrich), WASF1 (Upstate), HTT (Millipore) and β-actin (Sigma-Aldrich) used for protein level quantification and normalization following manufacturers’ protocols. Cells were seeded on 6-well plates, harvested and lysed with RIPA buffer (50 mM Tris-HCL, pH 7.4, 150 mM NaCl, 1mM EDTA, 1% NP-40, 1% sodium Deoxycholic acid, 0.1% SDS, protease and phosphatase inhibitors) 48 hours after transfection for co-immunoprecipitation. For V5-tagged protein immunoprecipitation, lysates were incubated with anti-V5 agarose affinity gel for 90 minutes and washed three times in RIPA buffer following the manufacturer’s protocol (Sigma-Aldrich). For GFP-tagged precipitation, lysates were pre-cleared with protein G Sepharose beads (GE Healthcare) for one hour, incubated overnight with GFP antibody (mouse, Covance), and incubated with protein G sepharose beads for one hour before 3 washes with RIPA buffer. All steps were performed at 4°C. Antibodies used for detection of tagged-proteins were anti-V5 (Invitrogen), anti-GFP (Cell signaling).

Immuofluorescence and filopodia counting - NIH3T3 were plated on poly-L-lysine 8-chambers slides (BD Biosciences). 48 hours after transfection, cells were rinsed once with 1X PBS and fixed with 4% PFA for 10 minutes and permeabilized with 0.2% Trion X-100 for 2 minutes. After 30 minutes of incubation in blocking solution (5% normal donkey serum in PBS), cells were incubated overnight at 4°C with anti-HTT (Millipore) and anti-V5 (invitrogen) primary antibodies. After incubation with respective secondary antibodies coupled with Alexa fluor 488 and 647 (Invitrogen) for one hour at room temperature, cells were incubated with Rhodamine-phalloidin coupled with Alexa fluor 555 (Invitrogen) for 20 minutes at room temperature in order to detect filamentous actin. Cells were washed three times with PBS and mounted using Prolong Gold with Dapi (Invitrogen). 15 cells per experiment were imaged using confocal microscope LSM 780 (Zeiss). Three replicates per condition were performed. Images were analyzed with Zeiss Zen (2011) software, V5- and HTT-positive cells were digitalized using ImageJ process ‘find edges’; number of actin-positive filopodia per cell was counted manually for each condition. Number of
filopodia per cell was expressed as relative value compared to average filopodia number in control cells. Filopodia were double counted by a blinded experimenter. T-test was used for statistical analysis (GraphPad prism). Co-localization experiments were performed using the same methods and material.

RESULTS

Network Properties of Huntingtin Interacting Proteins in HDNet

We had previously performed two large-scale screens to identify novel HTT interacting proteins using yeast two-hybrid (Y2H) and mass spectrometry-based methods, (16). For the Y2H screen, polyQ containing HTT-fragment baits of amino acids 1–90, 1–450 or 1–740 were screened in both wild-type (23Q) and mutant (>45Q) forms against multiple prey libraries generated from human cDNA. Stringent criteria have been used to define high-confidence core dataset comprised of 102 direct HTT interacting proteins (HIPs). We then extracted interaction data for these 102 HIPs to include secondary interacting proteins. This resulted in a network containing 2141 nodes: HTT, 102 primary and 2038 secondary interacting proteins connected through 3235 edges. These edges include 102 interactions of primaries with HTT, 51 primary-primary interactions, and 3082 primary-secondary interactions, representing data obtained from 9423 individual Y2H positive yeast colonies (Figure 1, Supplemental Tables S1 and S2). We refer to this network as HDNet.

HDNet is centered on HTT with two levels of partners and includes interactions between primary nodes (Figure 1B). Because we do not have information about interactions between secondary nodes, the network does not have a scale-free topology. In order to characterize the connectivity properties of HDNet members in the context of a scale-free network, we mapped HTT primary and secondary partners in the curated large-scale HPRD protein interaction network (HPRD Release 9, www.hprd.org) (23). We then calculated the shortest-path lengths among them, using the literature-curated interactions described in HPRD. With nodes defined according to Entrez Gene IDs, 79 out of 102 primary and 1408 out of 2038 secondary interacting proteins were present in the largest sub-network in HPRD, which consists of 8783 of the total 9019 proteins (Figure 2A).

This analysis showed that both primary and secondary proteins present in HDNet have significantly higher average node degrees as compared to the average for HPRD as a whole (18.25 and 10.80 vs. 7.63, both p-value < 0.001). This is true for median node degrees as well, suggesting that the high connectivity of HDNet proteins in HPRD is due to presence of highly connected outliers (Figure 2A and B). The average shortest path-lengths among both primary (3.39) and secondary HIPs (3.87) are significantly shorter than that observed in HPRD as a whole (4.25; Figure 2C and D; both p-value < 2.2e-16; t-test). The significantly higher connectivity observed among HDNet primary and secondary nodes within HPRD background suggests that these groups of proteins are enriched for shared functions and/or membership in common biological pathways (30). However, high node degrees could also contribute to short path-lengths between nodes. We thus analyzed the properties of randomly generated networks to determine whether this was the case. We first tested if the number of proteins in each group influenced the respective connectivity. We selected random cohorts of proteins with population sizes of HDNet primary (79) or secondary (1408) interactors from HPRD and calculated the average shortest-path lengths for these. This process was repeated $10^6$ times for the primary cohort simulation and $10^5$ times for the secondary cohort simulation. In both cases, the shortest path-length distributions are very similar to the HPRD as a whole, approximately 4.25 in both cases Data not shown). Given that nodes in HDNet have a higher average node degree as compared to HPRD, we wanted to determine how this property might contribute to the shorter path-lengths observed between primary and secondary HIPs in HPRD. To do this we repeated the simulation with randomly selected cohorts as described above but preserved the node degree distributions of the primary and secondary nodes. These simulations were performed $10^4$ times in each case. We observed that sub-networks simulated this way are less interconnected than either primaries or secondaries (Figure 2 C and D, respectively).

Analyses of the network properties of primary and secondary nodes in HPRD indicate...
demonstrate that HIPs identified in our Y2H screen tend to have higher nodes degrees and tend to be more highly interconnected. In both cases these differences are statistically significant. The high degree of interconnectedness of HDNet proteins in the independently derived and curated HPRD protein interaction network indicates that these proteins have shared properties and are likely to participate in shared biological functions. The increased node degree distribution observed among HIPs also indicates that the HTT network is enriched for highly connected proteins.

Functional Analysis of HDNet

Functional analysis of HDNet was performed using the Ingenuity Pathway Analysis (IPA) tool (Ingenuity System Inc, www.ingenuity.com). We found that 51 canonical pathways were significantly enriched (B-H p-value = or <0.01) among HDNet proteins (Table S3). The 20 most significant canonical pathways enriched in HDNet are shown in Figure 3A. Analyses were also performed separately with either primary or secondary nodes alone. This was done in order to determine the relative contribution of each category of partners (direct and indirect) to pathway enrichments, as well as to uncover potential bias due to an overlap between our dataset and HTT interacting proteins curated in the IPA knowledgebase. Notably, the forth most significantly enriched canonical pathway in HDNet is Huntington’s disease signaling. Some of the proteins in this pathway have been reported as HIPs and have been validated in multiple sources (16). Interestingly, HD signaling is also significantly enriched in secondary interaction partners. These results further validate the biological significance of HDNet.

The top three most significantly enriched pathways revealed by IPA analysis are related to protein synthesis and protein homeostasis: EIF2, EIF4, p70S6K and mTOR signaling pathways (Figure 3A). Primary HTT partners like PIK3R1, 2 and 3 (Phosphoinositide 3-Kinase Regulatory) subunits and EIF3A (Eukaryotic elongation Factor 3 member A), as well as secondary partners like EIF and ribosomal proteins S and L family members are represented in all these canonical pathways (Table S3). Of note, the primary HTT partners PIK3R family members are present in a number of enriched pathways. Dysregulation of the PI3K pathway is associated with numerous cancers (31), inflammatory diseases (32) and metabolic diseases like diabetes (33). This pathway has also been shown to play a critical role in neurodegenerative diseases such as HD (34,35).

Four other enriched canonical pathways are determined mainly by primary interacting partners: NRF2-mediated oxidative stress response, Aldosterone signaling in epithelial cells, Clathrin-mediated endocytosis signaling, and RAR activation. NRF2, aldosterone, and RAR signaling pathway findings highlight the transcripational role of HTT and its dysregulation in HD (36). The Clathrin-mediated endocytosis pathway is known to be affected by mutant HTT (37,38). Our data from primary and secondary interacting partners indicate that multiple components of these pathways interact with mutant HTT.

Enriched pathways driven by the secondary partners provide a broader view of global signaling pathways potentially impacted by HTT. These include: Rho Family GTPase, Actin cytoskeleton and ILK signaling as well as Molecular mechanisms of cancer. Interestingly, Rho family GTPases signaling is enriched in the secondary partner set, with Rho family, RHOA, and ARHGDI (RHOGDI), sharing components with actin signaling and ILK (integrin-linked kinase) pathways. These canonical pathways are all involved in cell migration, adhesion, proliferation and signal transduction (39,40). Members of ARHGEF (anti-Rho-guanine-nucleotide-exchange factor), Rho GTPases (ROCK1 and RAC1), myosins and WAS (Wiskott-Aldrich syndrome) protein families are found as secondary HTT partners. Several studies have showed that Rho GTPases, in particular ROCK1, have an effect on aggregation and degradation of mutant HTT (41,42). As other components of these pathways have been highlighted independently with our Y2H screen, we suggest that this could also support the known role of HTT in vesicle trafficking as well as novel functions and pathological effects on cell adhesion and cell migration.

Correlation of HDNet with Transcriptional Dysregulation in HD

Integration of data from different types of genome-scale approaches has been shown to facilitate functional annotation and to help to
formulate biological hypotheses (20,43). In order to prioritize the exploration of enriched pathways and associated protein networks, we next integrated HDNet pathway cohorts with HD gene expression data. We focused on gene expression dysregulation data in grade 2 HD patient caudate nucleus. (24,25). Because some canonical pathways had significant overlap in gene members, highly similar pathways were combined into composite lists prior to analysis. EIF2, EIF4/p70S6K and mTOR signaling (Figure 3A) were grouped into a pathway referred to as “mTOR EIF2 and EIF4” (Figure 3B). Similarly, Rho Family GTPase, RhoA and RhoGDI (Figure 3A) signaling were grouped into a pathway referred to as “Rho GTPase family (Figure 3B). We then tested whether canonical pathways identified with Ingenuity analysis and grouped pathways were enriched in dysregulated genes identified in HD caudate. Six canonical pathways were found enriched in dysregulated genes as compared to genome as well as to genes represented in the entire HDNet dataset (Figure 3B; hypergeometric test, B-H corrected p-value < 0.01 and Supplemental Table 4). Subnetworks of dysregulated proteins from enriched pathways are shown in Figure 4. These subnetworks show interconnections based on a combination of HDNet and HPRD data. Subnetworks from canonical pathways without significantly enrichment in genes dysregulated in HD caudate are shown Figure 5. Notably, in this analysis to identify HIPs with dysregulated expression, the Huntington’s disease signaling pathway showed the highest enrichment of dysregulated genes. This analysis highlighted also the involvement of Rho GTPases family signaling, with a group of proteins found in more than one enriched canonical pathway. These include EZR (Ezrin), BAIAP2 (Brain-specific Angiogenesis Inhibitor 1-Associated Protein 2), PI3K family members, CREB and CREBBP (Table S4 and Figure 4).

Based on a combination of pathway analysis combined with gene expression data from HD brain, we focused on the Rho GTPase family module. Rho GTPases are involved in numerous of cell processes acting downstream of growth factor receptor activation. Exchange of GDP for GTP by GEFs (guanine exchange factors) activates small GTPases and results in binding to their downstream effectors. GTP hydrolysis by GAPs (GTPase activating proteins) inhibits their function. RhoGDIs (Rho guanine nucleotide-dissociation inhibitors) regulate Rho GTPases activity by disassociating them from plasma membrane (44). Rho GTPases such as RAC1 or CDC42 activate WAVE complexes (nucleation-promoting factors, including WAS, WASL [N-WASP] and WAVE1-3 (WASF1)), which in turn activate Arp2/3 complex, leading to induction of actin polymerization and remodeling of cytoskeletal interactions. Formins, like mDia, protect barbed ends of actin filament from capping, promoting therefore elongation. Activated RAC1 binds to BAIAP2 to form a protein complex with WAVE to induce membrane ruffling and actin polymerization through Arp2/3 complex (45). Rac also activates PAK proteins, responsible of the delivery of WAVE protein to the plasma membrane. Ena/VASP complexes, anti-capping proteins, are found at the tip of filopodia. BAIAP2 induces filopodia formation through its I-BAR domain (see Table 1 and Figure 6), inducing cluster formation of PIP2 at the membrane and activating PIP2-binding proteins such as WASL (46). BAIAP2 activity is inhibited by threonine phosphorylation, leading to 14-3-3 protein binding (47).

EZR is a member of ERM (Ezrin, Radixin and Moesin) proteins. ERM proteins mediate the attachment of the membrane to actin filaments and are activated by phosphorylation. They are involved in membrane organization and signal transduction (48). Phosphorylated forms of ERM proteins are found at the site of filopodial shafts in axonal growth cones, and are dephosphorylated in response to signals that cause growth cone collapse and loss of filopodia (49).

**Domain Interactions in HDNet**

HDNet is derived from 9423 unbiased observations of individual binary protein interactions (i.e. Y2H positive yeast colonies). Because the bait and prey libraries used in this study were prepared from cDNA fragments, Y2H interactions reported here are between protein fragments. The average insert size in our Y2H libraries is 481 bp (+/- 140 bp). This corresponds to an average protein fragment size of 160 amino acids. We used CDD (conserved domains database) (50) to identify conserved domains contained in protein coding regions for both
primary and secondary HTT interacting partners. Of these, specific conserved protein domains could be identified in 6541 (35%) activation domain (AD) fusions and 3903 (21%) binding domain (BD) fusions. In 2268 (12%) cases conserved domains were identified for both AD and BD in a binary pair (Table S5). In order to characterize the domain-domain interaction information in HDNet, we determined frequency of domains present in the protein sequences mediating binary interaction with HTT. Table 1 shows the conserved domains most frequently present in primary partners interacting directly with HTT. Only those domains observed interacting with HTT two or more times are included. The WW domain was found as the most frequent conserved domain interacting with HTT. The WW domain is a short module of 40 amino acids that mediates protein-protein interaction with proline-rich regions (PRR) and thus likely to interact with the PRR of HTT (51). Binding of TCERG1, APBB members, WBP4, and SETD2 with HTT are known to involve WW-PRR domain interactions (11,52-54). It has also been shown that interactions involving the PRR of HTT can be affected by polyQ expansion (55), indicating potential roles in HD pathogenesis (56). The FF domain was also found at high frequency among primary interactors. Composed of three α-helices, FF domains are present in RNA regulatory proteins and Rho GTPase regulatory proteins, and often found adjacent to WW domains (57,58). High frequency of interactors with DNA-binding domains such as HLH (helix-loop-helix) and zinc-finger domains are consistent with the role mutant HTT in direct effects causing transcriptional dysregulation (36,59). SH2 and SH3 (Src Homology 2 and 3) domains were also found at high frequency in primary partners sequences responsible for their interaction with HTT. The SH2 domain is mainly found in adapter proteins and recognizes tyrosine-phosphorylated sites involved in intracellular signaling cascades (60). SH3 domains are also present in adapter proteins that link partners with their respective PRRs (61). Like the WW domain, SH3 has been implicated in HD pathogenesis through its interaction with the PRR of HTT. Two domain superfamilies are linked to protein degradation, DnaJ and UBA (Ubiquitin Associated) domains. Proteins containing these domains have been implicated in mutant HTT toxicity and clearance of HTT aggregates (62-64).

The presence of domains like RhoGAP and DHR2_DOCK (Dock Homology Region, a GEF domain) indicates a potential role of HTT in activity of small GTPases such as RAC1 or CDC42. BAR (Bin-Amphipysin-Rvs) domain superfamily is responsible for membrane remodeling, invagination or protrusion, in collaboration with nucleation promoting factors. BAR domain superfamily can be divided into 3 families, based on structure and functions: N-BAR and F-BAR domains form homodimers that bind concave membrane surface, creating a membrane tubular invagination, whereas the I-BAR domain is responsible for membrane protrusion (e.g. lamellipodia/filopodia) (65). These findings are in agreement with results showing enrichments for Rho family GTPases and Actin signaling pathways. This analysis adds a new insight into PPI data, more precise resolution for HTT protein interactions at pathway and network levels. As an example, we represented primary partners of Rho GTPases module (Figure 6), with their interactions with candidate conserved domains based on our domain analyses. Results showed that BAIAP2 seems to interact mostly through its SH3 domain with HTT. The BAR domain of BAIAP2 is a member of I-BAR, or IMD (IRSp53-MIM homology domain). BAIAP2 interacts through IMD with small GTPases (CDC42, RAC1, RHOF) and with downstream effectors like EPS8, Mena and WAVE family members through its SH3 domain for actin remodeling (45,66-70). Analysis of interactions of BAR domain of BAIAP2 highlighted PDZ domains present in MAGI (membrane associated guanylate kinase, WW and PDZ domain containing) and DLG (discs, large homolog), SH3 domain (present in EPS8), and RhoGEF domains (RASGRF1 and FGD5). This is in agreement with the role of BAIAP2 in modulating actin cytoskeleton and membrane protrusion through small GTPases signaling. Domain analysis shows that HTT interacts with ERM domain of EZR. We also note that all three members of PIK3R subunits interact with HTT through their first SH2 domain. These data support a model of HTT acting as a large scaffolding protein interacting with multiple components of signaling and structural regulators of cytoskeletal...
and membrane remodeling factors at sites of cell movement.

**Modifiers of HD Toxicity in the Rho GTPase Pathway**

Our group and others have shown that physical partners of HTT are good candidates as potential modifiers of polyglutamine toxicity (15,16,71). As a first step of functional analysis, we asked whether manipulating levels of components of the Rho GTPases subnetwork were modifiers of mutant HTT cytotoxicity. To do this, we used an assay based on immortalized mouse striatal cell Hdh<sup>7Q/7Q</sup> and Hdh<sup>111Q/111Q</sup> lines (29) to evaluate the effect of knock-down of candidates on mutant HTT-specific caspase activation upon serum withdrawal (28).

Because it is a significantly enriched canonical pathways with a high levels of transcriptional dysregulation (Figures 3 and 4), we decided to focus on components of the RhoGTPase signaling module for functional studies. This module contains seven primary HIPs (Figure 4). Of these, NFKB1 and CREBBP are known to be modifiers of HTT toxicity (72,73). These were not studied further. Of the remaining primary partners in this subnetwork, siRNA-mediated knockdown of three of the five tested proteins resulted in significant modification of toxicity in this cell model. Knock-down of BAIAP2 showed the most significant reduction of mutant HTT toxicity (Figure 7), without any effect on caspase activity in the Hdh<sup>7Q/7Q</sup> cell line (Figure 8). Intriguingly, expression of BAIAP2 is down-regulated in caudate of grade 0-2 HD patients. This could suggest a toxic role of the interaction between BAIAP2 and mutant HTT, with a compensatory effect of down-regulation in diseased neurons. Knock-down of EZR showed also a rescue effect on mutant HTT toxicity, without any effect on Hdh<sup>7Q/7Q</sup> cells. In this case, gene expression profiling of HD caudate showed an increase of EZR mRNA level, suggesting that expression of EZR could be detrimental for HD neurons. Knock-down of regulatory subunits of PI3K seemed to enhance mutant HTT toxicity, with a significant effect of PIK3R1 knock-down effect in Hdh<sup>111Q/111Q</sup> cells, without any effect on Hdh<sup>7Q/7Q</sup> cells.

EZR, BAIAP2, and PI3K proteins are keys components of signaling pathway through Rho family GTPases, but are also involved in actin cytoskeletal organization and are present in the IPA Actin cytoskeleton signaling pathway (Figure 4). We examined secondary partners that were common between these two modules and asked whether they could modulate mutant HTT toxicity in striatal cell lines. PAK2 (p21 activated kinase 2) is a serine/threonine kinase that links small GTPases CDC42 and RAC1 to cytoskeleton reorganization (74). siRNA-mediated knock-down of RAC1 and PAK2 showed significant reduction of mutant HTT toxicity, without any effect on caspase activity in the Hdh<sup>7Q/7Q</sup> cell line. Overall, the observed suppressor effect of knock-down of RAC1 and PAK2 (and WASF1 which trended toward suppression) confirmed the involvement of BAIAP2 and EZR in mediating mutant HTT toxicity.

We then examined the functional significance of the interaction between HTT and Rho GTPase pathway members. Out of the numerous cell processes requiring components of this signaling pathway, cell migration, cell adhesion, and neurite outgrowth were of particular interest in regard to HD. One of the first molecular steps for these cellular processes is structural reorganization of the plasma membrane. Various plasma membrane structures including invaginations (caveolae, clathrin-coated pits) and membrane protrusions, such as lamellipodia and filopodia, are involved in cell morphogenesis, endocytosis, cell migration and neuritogenesis. Filopodia are cylindrical extensions of the plasma membrane that contain bundles of parallel actin filaments at their core (75). Filopodia are thought to act as sensors, probing their microenvironment and regulating cell protrusion and cell migration in response to extracellular cues. Key components of focal adhesions, such as integrin, cadherin, PTK2 (FAK, Focal Adhesion Kinase) and Talin, are found in the tips of filopodia (76,77). These cytoskeletal-membrane structures are necessary for neurite formation and outgrowth in cortical neurons (78,79), as well as synaptogenesis (80). It has been shown that inhibition of Rac1 GTPase function decreases the number of axonal filopodia as well as at growth cone formation in sensory neurons, whereas inhibition of Cdc42 function inhibits filopodia formation only at growth cones (81). In these filopodial structures, BAIAP2 plays a central role as a linker between actin filaments,
through its IMD domain and plasma membrane (82) and has been shown to regulate filopodia formation (47).

We confirmed the binary physical interaction between BAIAP2 and HTT observed with Y2H using co-immunoprecipitation methods. Using HEK293T cells over-expressing an HTT fragment (1-558) fused to GFP and BAIAP2 fused to V5 tag, we observed co-immunoprecipitation of normal (23Q) and mutant (135Q) HTT with BAIAP2 (Figure 9A). We were also able to show a reciprocal co-immunoprecipitation of BAIAP2 with HTT fragment (Figure 9B). Moreover, using NIH-3T3 cells, we showed that both non-expanded and mutant HTT colocalized with BAIAP2, and filamentous actin at the tip of filopodial structures (Figure 9C).

We next investigated the function of HTT in filopodia formation in NIH-3T3 cell model. We co-transfected mouse fibroblasts with full-length HTT, normal (17Q) or mutant (138Q), or an empty vector control, along with V5-tagged BAIAP2, or V5-tagged LacZ (control). We observed that ectopic expression of BAIAP2 led to a significant increase in the number of actin-positive filopodia in NIH-3T3 cells when co-transfected with empty control. Overexpression of normal full-length HTT did not interfere with BAIAP2-induced increase of filopodia. However, expression of mutant HTT completely abolished this effect (Figure 10A). This result suggests that presence of mutant HTT inhibits the induction of filopodia formation by BAIAP2. Additionally, we showed that siRNA-mediated knock-down of BAIAP2 decreased number of filopodia-like protrusions in mouse embryonic fibroblasts, without any effect of normal HTT overexpression (Figure 10B). Presence of mutant HTT showed a trend to abolish this decrease. Filopodia are dynamic structures; elongation and retraction of the finger-like sensors are not yet completely understood. Rho Family members have been shown to be involved in both formation and stabilization of these structures, and it would be of great interest to study dynamics of filopodia in vitro and vitro, in particular at neuronal, axonal and synaptic level. These experiments demonstrate that huntingtin interacts both physically and genetically with the filopodial protein BAIAP2. Furthermore, mutant HTT can interfere with the ability of BAIAP2 to modulate filopodial morphogenesis.

**DISCUSSION**

Here we describe HDNet, a protein interaction network that provides a genome-scale resource for elucidating normal functions of HTT, novel pathogenic mechanisms of mutant HTT as well as deeper mechanistic insights into known pathways affected in HD. Partners of HTT identified in our screen, at both primary and secondary levels of interaction, showed enhanced interconnectivity as compared to other proteins in the context of HPRD, an independently derived and curated reference protein interaction network. We demonstrated that this connectivity is not due to node degree effects or annotation biases in HPRD. We conclude that independent validation of enhanced connectivity of HDNet arises from shared functional properties relevant to the functions of HTT as well as pathogenic process in Huntington’s disease.

HDNet is significantly enriched in proteins that function in pathways known to be involved in HD, such as protein homeostasis, cytoskeleton, vesicle trafficking (Figure 3). These pathways were recently discovered and described as preferentially enriched in HTT partners from two interaction screens by affinity purification followed by mass spectrometry from brain of HD mouse models (14,17). Here, we demonstrate that expanding the HD network with secondary interacting partners and combining enriched functional modules with gene expression dysregulation data from human brain tissue, highlights signaling by Rho family GTPases and actin remodeling as being processes in HTT function and HD pathogenesis.

HDNet is a protein interaction network defined at protein domain resolution. Identification of binding domain underlying protein interaction networks enhances our mechanistic understanding of how normal and pathological mechanisms may operate at the molecular level (83). This study used a Y2H method based on construction of binding domain and activation domain libraries from cDNA fragments. Therefore interactions reported here are between protein fragments rather than between full-length proteins (16,21). In many cases these fragments were shown to contain specific conserved protein domains (as defined by the
NCBI Conserved Domain Database). Knowledge of specific domain-domain interactions provides a higher resolution understanding of how specific proteins are interacting with HTT itself as well as HTT binding partners. With regard to HD pathogenesis, identification of binding domains of HTT partners is of high interest. Some studies suggest that the normal role of polyQ regions in proteins would be to stabilize PPI, and expansion of polyQ could have a deleterious effect through a gain of abnormal interactions or impairments of protein interaction dynamics (84). Recently, the role of the proline-rich region of HTT has been highlighted in different studies. Intrabodies targeting PRR were shown to reduce toxicity of mutant HTT and increase turnover of mutant HTT (85). It was also shown that deletion of the PRR domain in full-length HTT caused late onset learning and memory deficits in transgenic mice (86). This suggests that protein interaction with the PRR domain of HTT can affect both its normal and toxic activities as well as turnover. Partners of HTT containing SH3 or WW domains in the binding sequences could thus represent an interesting group of modifiers. We also note that a number of proteins with SH3 domains are involved in cell motility pathways. These are SRGAP1, SRGAP2, SRGAP3 and BAIAP2.

Based on analysis of canonical pathways represented in HDNet and gene expression changes observed in HD brain, we focused on RhoGTPase signaling to validate a specific functional module as being involved in HTT function and toxicity. The potent loss-of-function suppression of HTT toxicity observed in STHdh^{111Q/111Q} cells upon knock-down of BAIAP2 further focused our attention on filopodia components as modifiers of mutant HTT toxicity in cell models. These structures are essential for cell adhesion, cell migration and neurite outgrowth (87). Here we show that expression of mutant HTT impairs BAIAP2-induced filopodia formation. This phenomenon is observed for induction of filopodia formation following BAIAP2 overexpression, as well as a decrease of formation of these structures following knock-down of BAIAP2. This suggests that mutant HTT can impair of dynamic regulation of filopodia by BAIAP2. The regulation of BAIAP2-induced filopodial formation remains to be elucidated, as well as the specific involvement of this process in neuron-specific pathways. BAIAP2 is also a binding partner of PDZ-domain-containing proteins such as DGL-4 (PSD-95) in the postsynaptic density indicating that the HTT-BAIAP2 interaction may influence both pre- and post-synaptic processes (88,89). BAIAP2 has also been shown to interact with ATN1 (atrophin-1), protein responsible for DRPLA, a polyQ expansion disease with pathologic and clinical similarities to HD (90). Identifying the protein composition of filopodia neurons affected in HD, and deciphering the involvement of HTT in these dynamic structures could help elucidate novel mechanisms of neuronal dysfunction. In human brain, previous studies showed marked morphological changes in dendritic structures and branching of medium spiny neurons in post-mortem HD brain (91,92). Our data indicate that dynamic organization of neuronal processes as well as sites of cell-cell contacts may be a primary pathogenic mechanism in HD. Furthermore we provide evidence that components of the Rho GTPase signaling cascade such as BAIAP2 can be directly affected by mutant HTT and are therefore candidates for HTT mediated defects in cell morphology. The protein machinery regulating cytoskeletal-membrane interactions and filopodia formation represent key targets for further studies with regard with neurodevelopmental pathways and synaptic homeostasis in Huntington’s disease.

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ABBREVIATION LIST

AD, activation domain; BD, DNA binding domain; HD, Huntington’s disease; HIP, huntingtin interacting protein; HPRD, Human Protein Resource Database; IPA, Ingenuity Pathway Analysis; Y2H, yeast two-hybrid

FIGURE LEGENDS

**FIGURE 1. HDNet is an HTT Interactome Comprised of Primary and Secondary Interacting Proteins.**
(A) The network is composed of HTT (red), 102 primary partners and 2038 secondary partners connected though 3235 edges. (B) First level of HTT interacting proteins shows HTT-primary and primary-primary interactions. Node colors represent gene expression dysregulation in HD human caudate sample.

**FIGURE 2. Connectivity Properties of HDNet.**
(A) Distribution of node degrees of HDNet partners in the HPRD network. (B) Average node degrees of primary and secondary partners compared HPRD nodes in HPRD network (with average, SD and median, p-value<0.001). (C) Distribution of average shortest path-lengths from HPRD subnetworks with the same node degree distribution as primary partners (red line indicates average shortest path-length between
primary nodes). (D) Distribution of average shortest path-lengths from HPRD subnetworks with the same node degree distribution as secondary partners (red line indicates average shortest path-length between secondary nodes).

FIGURE 3. Functional Characterization of Primary and Secondary HTT Interaction Partners Using IPA.
(A) Ingenuity Pathway Analysis of HDNet members, and primary and secondary separately. The 20 most enriched canonical pathways are represented based on whole dataset ranking. Red line indicates threshold for significance (B-H corrected p-value=0.05). (B) Enrichment analysis of IPA functional subgroups for gene expression dysregulation from human HD caudate (log2 fold-change is indicated; *B-H corrected p-value<0.01).

FIGURE 4. Modules Identified by Functional and Gene Expression Enrichment Analysis.
Functional groups identified using Ingenuity Pathway and enriched gene expression dysregulation analyses are represented as modules based on a combination of HDNet and HPRD interactions. Node shape indicates the interaction type (diamond, primary or hexagon, secondary) with HTT from HDNet data. Node color indicates dysregulation in gene expression (log2 fold change is indicated; yellow, increased and blue, decreased). Solid lines indicate interaction from HDNet. Dashed lines indicate interactions from HPRD.

FIGURE 5. Additional Functional Modules Identified by Ingenuity Pathway Analysis.
Proteins identified using IPA Canonical Pathways, but not significantly enriched in gene expression dysregulation are represented as modules based on a combination of HDNet and HPRD interactions. Node shape indicates the interaction type (diamond, primary or hexagon, secondary) with HTT from HDNet data. Node color indicates dysregulation in gene expression (log2 fold change is indicated; yellow, increased and blue, decreased). Solid lines indicate interaction from HDNet. Dashed lines indicate interactions from HPRD.

FIGURE 6. Domain-domain Interactions of Primary Partners in the Rho signaling module.
Schematic representation of primary partners of HTT from Rho GTPases signaling pathway. Rectangles and associated labels indicate conserved domains identified from Y2H sequence analysis. Horizontal black lines indicate the coverage of the domain by positive clone sequences; numbers to right of black lines and thickness indicate the number of independent Y2H observations. Arrows indicates interactions with other conserved domains (vertical labels) contained in interacting proteins of family members (horizontal labels). Red lines indicate coverage of positive sequence for HTT interaction regions. Thickness and numbers to right indicate number of independent observations of Y2H interaction of protein with HTT.

FIGURE 7. Rho Family GTPase Signaling Components Modify Mutant HTT-Induced Toxicity.
Caspase 3/7 activity following siRNA-mediated knock-down of Rho family GTPase signaling components in STHdh111Q/111Q cells (n=3, *p-value<0.05, **p-value<0.01, t-test). Data are represented as relative value to STHdh111Q/111Q treated with Non-Targeting siRNA (NT). Percent protein knock-down and standard deviations as determined by western blot (n=3) are indicated.

FIGURE 8. Modifier Effects of Rho Family GTPases Signaling Components is Specific to Mutant HTT-induced Toxicity.
(A) Caspase 3/7 assay following siRNA-mediated knock-down of Rho family GTPase signaling components in STHdh111Q/111Q cells (n=3). Data are represented as relative to STHdh111Q/111Q treated with Non-Targeting siRNA (NT). Percent protein knock-down and standard deviations as determined by western blot (n=3) are indicated. (B) Caspase assay following BAIAP2 knock-down in STHdh111Q/111Q cells using
individual siRNAs from deconvoluted pools (n=3, *p-value<0.05, t-test). Percent protein knock-down and standard deviations as determined by western blot (n=3) are indicated.

FIGURE 9. HTT and BAIAP2 Interact and Co-localize in Cells.
(A) Co-immunoprecipitation of GFP-tagged HTT fragments with V5-tagged BAIAP2 expressed in HEK293T cells. BAIAP2 was immunoprecipitated with anti-V5 and HTT was probed with a GFP antibody. A lysate transfected with empty vector was performed in parallel to confirm a specific protein interaction. 10% of input for each protein lysate (left) and immunoprecipitation results (right) are shown for each protein. (B) Reciprocal co-immunoprecipitation of V5-tagged BAIAP2 with GFP-tagged HTT. HTT was immunoprecipitated with anti-GFP. A Native IgG pull-down was performed as a control (NS). (C) Colocalization (left panel) in actin-positive (red, center left) filopodia of BAIAP2 (purple, center right) and HTT (green, right) in NIH-3T3 cells. Nuclei were labeled with DAPI (blue).

FIGURE 10. Mutant HTT Interferes with BAIAP2-induced Filopodia Formation.
(A) NIH-3T3 cells were co-transfected with V5-tagged BAIAP2 or V5-tagged LacZ (control) along with full-length HTT (17Q), or HTT (138Q) or with an empty vector (control). Cells were fixed and stained with rhodamine-phalloidin, anti-V5 and anti-HTT. The number of actin-positive filopodia were counted in V5 and in HTT positive cells, 15 cells per experiment with n=3 (*p-value<0.05, t-test). The relative numbers of filopodia in each condition are indicated. (B) NIH-3T3 cells were co-transfected with BAIAP2 siRNA or Non-Targeting siRNA (control) along with full-length HTT (17Q), or HTT (138Q), or with an empty vector as (control). Cells were fixed and stained with rhodamine-phalloidin and anti-HTT. Number of actin-positive filopodia were counted in HTT positive cells, 15 cells per experiment with n=3 (*p-value<0.05, **p-value<0.01, t-test). The relative numbers of filopodia in each condition are indicated. (C-D) Expression levels of BAIAP2 and HTT were analyzed by immunoblotting with indicated antibodies, using β-actin as a loading control, for overexpression (C) and knock-down (D) of BAIAP2. (E-F) Representative images of rhodamine-phalloidin labeled cells co-transfected with BAIAP2 and HTT 17Q (E) or 138Q (F), and their respective digitized images.
| Domain                        | Accession  | N  | Associated Proteins (Gene_Symbol)                                      |
|-------------------------------|------------|----|-----------------------------------------------------------------------|
| WW superfamily                | cl00157    | 1325 | APBB2, TCERG1, WBP4, FNBP4, SETD2, WAC, PRPF40A                        |
| FF superfamily                | cl02610    | 170  | TCERG1, PRPF40A                                                       |
| zf-H2C2_2 superfamily         | cl16282    | 144  | SP3, KLF11, ZNF655, ZNF675                                           |
| SH2 superfamily               | cl15255    | 132  | PIK3R1, PIK3R2, PIK3R3                                                |
| SH3                           | cd00174    | 123  | PRMT2, PTK6, SRGAP3, BAIAP2, SORBS1, SRGAP2, OSTF1, SRGAP1            |
| Dynamitin                     | pfam04912  | 50   | DCTN2                                                                 |
| NrdE_NrdA                     | TIGR02506  | 46   | USP9X                                                                 |
| DnaJ                          | cd06257    | 43   | DNAJC11, DNAJC4, DNAJA3, DNAJC21                                     |
| UBA superfamily               | cl00153    | 42   | UBAC1, NUB1, OPTN                                                      |
| Pyruvate_Kinase superfamily   | cl09155    | 24   | PKM                                                                   |
| NR_DBD_like superfamily       | cl02596    | 21   | PPARG                                                                 |
| Snf7 superfamily              | cl02305    | 21   | CHMP4B                                                                |
| ARS2 superfamily              | cl04863    | 17   | SRRT                                                                  |
| ANK                           | cd00204    | 16   | OSTF1, NFKB1                                                         |
| RhoGAP superfamily            | cl02570    | 16   | ARHGAP24, ARHGAP25, SRGAP1                                           |
| DHR2_DOCK superfamily         | cl06123    | 14   | DOCK9, DOCK11                                                        |
| 14-3-3 superfamily            | cl02098    | 12   | YWHAB                                                                |
| ARM                           | cd00020    | 12   | KPNA3                                                                |
| Mre11_DNA_bind superfamily    | cl04421    | 12   | MRE11A                                                               |
| zf-C2H2_jaz superfamily       | cl09952    | 11   | DNAJC21                                                              |
| BAR superfamily               | cl12013    | 10   | BAIAP2, PACSIN1                                                      |
| TBD superfamily               | cl15118    | 10   | TANK                                                                 |
| DDT superfamily               | cl02674    | 7    | BAZ1A                                                                |
| VHS_ENTH_ANTH superfamily     | cl02544    | 7    | HIP1                                                                 |
| P4Ha_N superfamily            | cl07084    | 6    | P4HA1                                                                |
| PH-like superfamily           | cl00273    | 6    | DNM2, DNM1                                                           |
| SAM_superfamily               | cl15755    | 6    | SASH1                                                                |
| TACC                          | pfam05010  | 6    | TACC1                                                                |
| FEZ superfamily               | cl06682    | 5    | FEZ1                                                                 |
| zf-CCCH superfamily           | cl11592    | 5    | MKRN2                                                                |
| MT superfamily                | cl15084    | 4    | DYNC1H1                                                              |
| vWFA superfamily              | cl00057    | 4    | PSMD4                                                                |
| API5 superfamily              | cl09392    | 3    | API5                                                                 |
| HLH                           | cd00083    | 3    | SREBF2                                                               |
| KRAB_A-box superfamily        | cl02581    | 3    | ZNF133                                                               |
| Med15                         | pfam09606  | 3    | MED15                                                                |
| Adaptn_N                      | pfam01602  | 2    | AP2A2                                                                |
| BTB superfamily               | cl02518    | 2    | ZBTB16                                                               |
| Coatamer_beta_C superfamily   | cl06658    | 2    | COPB1                                                                |
| CULLIN superfamily            | cl10551    | 2    | CUL5                                                                 |
| ERM                           | pfam00769  | 2    | EZR                                                                  |
| Gene Name                  | ID      | Description                          | superfamily     | ID       | Description          |
|---------------------------|---------|--------------------------------------|-----------------|---------|----------------------|
| NGN_Euk                   | cd09888 |                                      |                 | 2       | SUPT5H               |
| PRP40                     | COG5104 |                                      |                 | 2       | PRPF40A, TCERG1      |
| pyrophosphatase superfamily | cl00217 |                                      |                 | 2       | PPA2                 |
| RING superfamily          | cl15348 |                                      |                 | 2       | MKRN2                |
| TPR                       | cd00189 |                                      |                 | 2       | GTF3C3               |
Fig. 2

|          | HPRD | HDNet-1° | HDNet-2° | total |
|----------|------|----------|----------|-------|
| Nodes    |      | 79       | 1408     | 9019  |
| Edges    |      | 1439     | 13337    | 34409 |
| Average  |      | 18.39    | 10.8     | 7.63  |
| SD       |      | 16.21    | 7.15     | 9.7   |
| Median   |      | 14.62    | 7.78     | 3.42  |

**B**

Node Degree

- HDNet-1°
- HDNet-2°
- HPRD

**C**

Probability Density

Average Shortest Path Length

**D**

Probability Density

Average Shortest Path Length
Fig. 6

BAIAP2

EZR

PIK3R1, isoform 1

SH3  RhoGAP  SH2  COG4942  SH2

PIK3R1, isoform 2

SH2  COG4942  SH2

PIK3R1, isoform 4

SH2  COG4942  SH2

PIK3R2

SH3  RhoGAP  SH2  SMC_prok_A  SH2

PIK3R3

SH2  Smc  SH2

NFkB1

RHD_n  IPT  ANK  Death

by guest on March 23, 2020
http://www.jbc.org/Downloaded from
Caspase-3 activity

dRFU/min/mg proteins relative to 111Q NT

% of knock-down: 42 94 54 83 45 32 41 41

(SD) (24) (3) (12) (15) (30) (9) (28) (18)
A Large-scale Huntingtin Protein Interaction Network Implicates Rho GTPase Signaling Pathways in Huntington's Disease
Cendrine Tourette, Biao Li, Russell Bell, Shannon O'Hare, Linda S. Kaltenbach, Sean D. Mooney and Robert E. Hughes

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