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Review

Cerebellar ataxia and functional genomics: Identifying the routes to cerebellar neurodegeneration ☆

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ABSTRACT

Cerebellar ataxias are progressive neurodegenerative disorders characterized by atrophy of the cerebellum leading to motor dysfunction, balance problems, and limb and gait ataxia. These include among others, the dominantly inherited spinocerebellar ataxias, recessive cerebellar ataxias such as Friedreich's ataxia, and X-linked cerebellar ataxias. Since all cerebellar ataxias display considerable overlap in their disease phenotypes, common pathological pathways must underlie the selective cerebellar neurodegeneration. Therefore, it is important to identify the molecular mechanisms and routes to neurodegeneration that cause cerebellar ataxia. In this review, we discuss the use of functional genomic approaches including whole-exome sequencing, genome-wide gene expression profiling, miRNA profiling, epigenetic profiling, and genetic modifier screens to reveal the underlying pathogenesis of various cerebellar ataxias. These approaches have resulted in the identification of many disease genes, modifier genes, and biomarkers correlating with specific stages of the disease. This article is part of a Special Issue entitled: From Genome to Function.

1. Introduction

The cerebellar ataxias are a group of clinically homogeneous and genetically heterogeneous neurodegenerative disorders, all characterized by progressive atrophy of the cerebellum and a clear loss of Purkinje cells, leading to impairment of motor function, balance, gait and speech. The most prominent clinical feature is cerebellar ataxia, which is often associated with additional neurological manifestations such as pyramidal, extrapyramidal and cognitive dysfunction. Given the clear overlap in disease phenotypes and the various modes of presentation of cerebellar ataxia, making a correct diagnosis is challenging. The disease inheritance patterns can be autosomal dominant, recessive, X-linked or even mitochondrial in a few ataxia syndromes. The precise number of cerebellar ataxias is unknown, but at least 37 dominantly inherited spinocerebellar ataxias (SCAs), 20 recessive ataxias and a few X-linked and mitochondrial inherited forms of cerebellar ataxia are known [1–3]. Taken together, all ataxias have an estimated prevalence of 15–20:100,000 [4]. Despite all the known disease-causing genes, around 30% of all cerebellar ataxia patients remain genetically undiagnosed.

In addition to a genetically heterogeneous background, a broad range of mutation types have been identified that contribute to the complex etiology of the cerebellar ataxias. A large number are caused by coding polyglutamine (CAG; polyQ) repeat expansions, or non-coding CTG, CAG, and GAA repeats, but cerebellar ataxias caused by penta- or hexanucleotide repeat expansions have also been reported [5]. Missense mutations, deletions, duplications, splice and truncating mutations have also been identified. All ataxia genes in dominant cerebellar ataxias seem functionally different but operate in shared pathways including protein misfolding and aggregation, impairment of the protein quality control system, dysregulation of gene transcription, RNA toxicity, and alterations in synaptic transmission [6,7]. On the other hand, alterations in mitochondrial functioning, DNA repair efficiency, synaptic transmission, chaperone activity, and metabolic functioning underlie recessive cerebellar ataxias [2]. The main challenge for clinicians and researchers is the development of a therapy that can intervene with these various disease mechanisms, as there is no therapy that slows progression or prevents these diseases from occurring.

In this review, we discuss how advances in technology allow us to reveal the whole-exome variation, and the whole-genome transcriptome or epigenome in human, cell and animal models of cerebellar ataxia (overview in Table 1). To get more in-depth information on the underlying genetic variation in the genome and gene expression associated with cerebellar ataxia, work using next generation sequencing, gene expression profiling, miRNA profiling and methylation profiling is reviewed. An additional method to examine the active transcriptome is ribosomal trapping, however, to date, this technique is not utilized in

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Table 1
Overview of all cerebellar ataxias used in this review.

Disease	Gene	Mutation type	Reference
Conventional mutations			
SCA5	*SPTBN2*	Missense	[17]
SCA19/22	*KCND3*	Missense/in frame deletion	[8,9]
SCA28	*ALG3L2*	Missense	[24]
SCA35	*TGM6*	Missense	[7]
SCA26	*eEF2*	Missense	[10]
Adult-onset, recessive spinocerebellar ataxia with psychomotor retardation	*SYT14*	Missense	[11]
Early-onset cerebellar neurodegeneration	*UCHL1*	Missense	[12]
Recessive cerebellar ataxia with spasticity	*GBA2*	Missense	[13]
X-linked cerebellar ataxia	*PMCA3*	Missense	[14]
X-linked cerebellar ataxia	*GJB1*	Missense	[15]
Cerebellar neurodegeneration and ataxia in mice	*RNU2*	Missense	[18]
Polyglutamine repeat expansions			
SCA1	*ATXN1*	CAG expansion	[27,28,30,36,62,67]
SCA2	*ATXN2*	CAG expansion	[29]
SCA3	*ATXN3*	CAG expansion	[31,64,65]
SCA7	*ATXN7*	CAG expansion	[28,32,43,66]
SCA17	*TBP*	CAG expansion	[68]
GAA repeat expansion			
Friedreich's ataxia	*FXN*	GAA expansion	[22,23,25]

ataxia research, and will not be discussed in this review. We examine how functional genomics leads to identification of the routes to neurodegeneration and improves our understanding of the pathogenesis of cerebellar ataxia.

2. Identification of novel cerebellar ataxia genes by next generation sequencing

The way in which genes for Mendelian disorders such as cerebellar ataxia are identified has changed significantly with the introduction of genome-wide genotyping and next generation sequencing. The traditional approach, first mapping the disease gene using linkage analysis in large pedigrees followed by candidate gene Sanger sequencing, was time consuming, labor intensive, and had low success rates. Furthermore, large pedigrees were rarely available for late age of onset diseases like the dominant cerebellar ataxias, the numbers of genetic markers were relatively limited, and the identified linkage intervals were large and contained many genes, which affected the time and costs necessary to study a single family.

Genome-wide genotype analysis using arrays containing up to a million single-nucleotide-polymorphisms (SNPs) to pinpoint candidate regions in the genome and whole-exome sequencing or sequencing of custom captured genomic regions to unravel millions of coding DNA fragments at the same time have had a great impact on disease gene identification in Mendelian disorders, as these approaches require smaller families, and have shorter turn-around times. In contrast to earlier methods, this approach does not always lead to the identification of the disease gene because approximately 20,000 variants will be identified per individual per sequence run, and the mutation could be located in the non-coding part of the genome. To be able to identify *the* causal variation, proper validation is needed by co-segregation analysis within additional family members (if available) and by the generation of functional disease models.

The first success story using this work scheme for dominant cerebellar ataxia was reported by Wang et al. [8], who identified heterozygous mutations in *TGM6* encoding transglutaminase 6 to underlie SCA35. Similarly, the voltage-gated potassium channel *KCND3* was identified as the SCA19/22 disease gene [9,10], and heterozygous mutations in *eEF2* encoding for Eukaryotic Translation Elongation Factor 2, were proven to cause SCA26 [11]. The recessive ataxias are very suitable for homozygosity mapping using high-density SNP arrays in affected individuals, which pinpoints a potential causal variant in one of the homozygous genomic regions, followed by whole-exome sequencing of two affected family members. Recently, synaptotagmin 14 (*SYT14*) was linked to adult-onset, recessive spinocerebellar ataxia with psychomotor retardation [12], ubiquitin carboxyl-terminal esterase L1 (*UCHL1*) to early-onset cerebellar neurodegeneration [13], and glucosidase beta 2 (*GBA2*) to recessive cerebellar ataxia with spasticity [14]. Additionally, sequencing using an X-chromosome capturing array or X-chromosome haplotype analysis followed by whole-exome sequencing identified two novel X-linked ataxia disease genes, *PMCA3* and *GJB1*, encoding for ATPase Ca2 + transporting plasma membrane 3 and gap junction protein beta 1, respectively [15,16].

Sequencing of RNA transcripts is mainly applied to count their abundance, but can also be used to identify novel splice variants and causal variations. As yet, no successes have been reported for humans. However, genome-wide RNA sequencing facilitated the identification of the disease gene, beta-spectrin (*SPTBN*), for canine cerebellar cortical degeneration [17], previously associated with SCA5 (*SPTBN2*) [18]. Likewise, a mutation in *U2* snRNA (*RNU2*) was shown to cause global disruption of alternative splicing and cerebellar neurodegeneration and ataxia in mice [19].

Over the last three years, nine novel cerebellar ataxia genes have been identified using a combination of gene mapping and next generation sequencing. These findings emphasize the genetic heterogeneity of these disorders and the complexity of the underlying disease etiology. The Gene Annotation Tool to Help Explain Relationships (GATHER) [20] showed that five out of these ten genes fit into the GO ontology category "transport" and/or "establishment of localization" (data not shown). Additionally, the Allen Brain Atlas (http://www.brain-map.org/) was used to determine the gene expression patterns in cerebellum and Purkinje cells. For six out of ten genes, moderate to high expression was seen in both granular cells and Purkinje cells (data not shown). In contrast, *UCHL1* and *GJB1* showed almost no expression in Purkinje cells but were expressed in the granule cells. However, a more detailed analysis revealed a clear distinction between the functions of these genes, as some operate in ion-transport, whereas others are involved in synaptic transport or maintain the proper localization of glutamate transporters [21,22]. Thus, despite the discovery of novel disease genes, more effort is required to reveal the pathways of

neurodegeneration and cerebellar ataxia, as there is no proper disease model available yet for most ataxias that could be used to study the causal molecular mechanism.

3. Genome-wide expression profiling in cerebellar ataxia

The application of expression profiling in neurodegenerative diseases relies largely on the ability to use post-mortem brain material, but this is only rarely available for rare cerebellar ataxias. Moreover, the collected brain material often represents the end stage of disease, so the resulting data is polluted with possible post-mortem effects, and the brain tissue homogenates often contain a mixed population of neurons that can each reflect a different pathophysiology. Single cell laser microscopy isolating only the Purkinje cells from the cerebellum may overcome this problem. Alternatively, blood or induced pluripotent stem cells (iPS) can be used that may reflect the pathology in the cerebellum of adult cerebellar ataxia patients. In the absence of patient material, *in vivo* or *ex vivo* models that express the disease genes and mutations of interest can be used, and we will focus on studies performed in humans and mice.

3.1. Gene expression profiling

To date, only three studies involving gene expression profiling of human blood samples in cerebellar ataxia have been reported, and one study using patient-derived iPS cells [23–26]. In blood from Friedreich's ataxia (FRDA) patients, differential gene expression was identified in genes expressed in the brain cortex and heart atria, as well as in biological processes such as mitochondrial fatty acid beta-oxidation, reactive oxygen species, and genotoxic stress response. This finding was strengthened by direct evidence of mitochondrial and nuclear DNA damage in the blood [23]. Coppola et al. [24] and Ku et al. [26] also detected dysregulation of genes involved in DNA repair in peripheral blood mononuclear cells (PBMCs) and iPS of cases, supporting the hypothesis that DNA repair is important in the etiology of FRDA. Surprisingly, the GO categories of genes that were differentially expressed in iPS showed marked overlap with the GO categories obtained during blood cell profiling, indicating that PBMCs can be a good alternative when iPS are not available. In line with this, gene expression profiling in lymphocytes of SCA28 patients revealed that the differentially expressed genes clustered in well-defined functional categories such as regulation of cell proliferation, regulation of programmed cell death, and response to oxidative stress [25]. Functional validation showed cellular metabolic deficits including alterations in mitochondrial fission/fusion leading to markedly reduced cell viability in SCA28 lymphocytes, corroborating previous work in which haploinsufficiency of *ALG3L2* led to mitochondria-mediated Purkinje cell dark degeneration [27].

Six studies used cerebellar tissue of transgenic, knock-out or knock-in mice for polyQ SCA types including SCA1, 2, 3 and 7 [28–33]. One of these studies identified common transcriptional differences between SCA1 and SCA7 in early disease stages, in addition to the many unique differentially-expressed genes for each disease model [29]. Genes involved in mRNA processing and chromatin were differentially expressed for SCA1, whereas transcription factor binding and MHC Class 1 proteins were identified for SCA7. Igfbp5, an insulin-like growth-factor binding protein, was found to be the most significant differentially expressed gene in both SCA1 and SCA7 cerebella that was coupled with increased activation of the insulin growth-factor 1 receptor, IGF1R. This work clearly showed that dysregulation of the IGF pathway is a common pathogenic response in SCA1 and SCA7. Serra et al. [28] used a strategy that led to the identification of a restricted set of genes that were differentially expressed specific to the development of SCA1. By comparing two non-ataxic transgenic SCA1 mouse lines with ataxic SCA1 mice, only 9 genes were identified that were differentially expressed in 5- and 12-week old ataxic mice. The functions of these genes revealed that the SCA1 Purkinje cell pathology could be caused by alterations in glutamate signaling. Defects in glutamate signaling due to mutations in glutamate transporters or receptors were proven to cause Purkinje cell degeneration and ataxia in mice and humans [34,35], demonstrating the importance of neuronal glutamate homeostasis in cerebellar functioning.

Transcriptional dysregulation in SCA1 might be caused by a partial loss-of-function of ataxin-1, as was proven by comparing transcriptional defects in ataxin-1 knock-out mice *versus* ataxin-1 knock-in mice [31], as many of the shared transcriptional changes were acting in identical directions in both mouse models. Notably, this work was unable to confirm the outcomes of a previous study reporting transcriptional changes in the ataxin-1 knock-out mice [36], with the exception of some genes. However, this could be due to differences in genetic background of the two mouse strains, the differences in age of the mice, and the type of microarray platform used. Ataxic *staggerer* mice that carry a spontaneous loss-of-function mutation in the gene encoding the transcription factor Rorα exhibit marked overlap in transcriptional profiles with ataxin-1 knock-out mice [37], including genes involved in calcium and glutamatergic signaling such as *Itpr1* and *Grm1*, in which loss-of-function mutations were reported, causing cerebellar ataxia in humans and/or mice [38,39]. This raises the opportunity of using the outcomes of transcriptional profiling to pinpoint novel disease candidate genes because down-regulation of these genes could contribute to ataxic phenotypes. Mice expressing mutant ataxin-3 exhibited reduced transcript levels of genes involved in glutamatergic neurotransmission, intracellular calcium signaling or MAP kinase pathways, $GABA_{A/B}$ receptor subunits, heat shock proteins and transcription factors regulating neuronal survival and differentiation. Additionally, upregulation of Bax, cyclin D1, and CDK5-p39 genes contributing to neuronal death, were also observed mutant ataxin-3 mice [32].

Alterations in the expression of transcripts involved in glutamatergic transmission, signal transduction, myelin formation, deubiquitination, axon transport, neuronal differentiation or glial functions and heat shock proteins were found to underlie the SCA7 pathology [33]. In contrast, only FBXW8, encoding one of the subunits of the ubiquitin ligase complex, seems to play a specific role in the cerebellar pathology of old ataxin-2 knock-in mice [30]. Depletion of soluble PABPC1 levels by sequestering in the mutant ataxin-2 aggregates might result in impaired RNA processing as well as deficient protein synthesis in SCA2 [40]. These two themes are familiar in ataxia because of the disease genes identified for SCA26 and SCA36, in which mutations in *eEF2* and *NOP56* cause defects in protein synthesis and RNA processing, leading to Purkinje cell loss and spinocerebellar ataxia [11,41].

One paper described the global gene expression profiles for various tissues such as liver, skeletal muscle and heart from a hypomorphic presymptomatic mouse model for FRDA [42]. The basic pattern of transcriptional dysregulation that was seen in skeletal muscle and liver matched their roles in energy metabolism including dysregulation of the PPARγ/PGC1A pathway by marked downregulation of *Pgc1a*, which is involved in mitochondrial biogenesis, energy substrate and utilization, and oxidative metabolism [43]. Alteration in this pathway may underlie the vulnerability of FRDA patients to diabetes as it also regulates the insulin response. Additionally, in heart muscle, a clear fiber-type switch and dysregulation of contractile proteins was observed that could predispose patients to heart defects, as are seen in many FRDA patients.

Recently, a comparative gene expression profiling experiment was performed in Purkinje cells isolated from mouse cerebella using laser capture microdissection from two different polyQ-disease mouse models, namely Huntington's disease and SCA7 [44]. This work revealed shared molecular abnormalities in Purkinje cells from both mouse models and indicated Pcp2-promoter repression as an early event in the polyQ disease pathogenesis. Additionally, the expression of two other genes, *Aldoc* and *Plcb3*, was also reduced in both models. This data indicates that glycolysis and inositol 1,4,5-trisphosphate and 1,2-

diacyl-glycerol production are necessary for proper Purkinje cell functioning. The 1,2-diacyl-glycerol activates protein kinase C gamma, (PKCg), in which mutations were found to cause SCA14 [45]. This is yet another example that highlights the strength of transcriptional profiling in elucidating the underlying routes to cerebellar neurodegeneration.

3.2. miRNA profiling

Over the last decade, the role of microRNAs (miRNAs) in regulating gene and protein expression has been established. Compelling evidence has accumulated that these 21-nucleotide long, non-coding RNAs play an important role in neuronal differentiation, as these molecules are markedly differentially expressed during brain development and are involved in dendritic spine formation and neurite outgrowth [46,47].

Schaefer et al. [48] demonstrated that the presence of miRNAs are essential for the survival of post-mitotic neurons such as Purkinje cells. Selective depletion of miRNAs in Purkinje cells by using conditional knock-out mice lacking the major miRNA-generating enzyme, Dicer, led to cerebellar neurodegeneration and ataxia. This data suggested an essential function for miRNAs in the etiology of cerebellar neurodegenerative disorders such as the spinocerebellar and cerebellar ataxias.

To date, only four studies have reported on the involvement of specific miRNAs in the neuropathology of dominant spinocerebellar ataxias, including SCA1 and SCA3 [49–52]. Direct binding of miR-19, miR-101 and miR-130 to the 3′-UTR of ataxin-1 was found to suppress the translation of the ataxin-1 protein [49]. Expression of these miRNAs suppressed mutant ataxin-1-induced toxicity and *vice versa*, knock-down of the miRNAs or mutagenesis of the miRNA-binding sites in the 3′-UTR elevated ataxin-1 toxicity in cell systems. Additionally, miRNA profiling of human brain samples revealed that, among others, miR-144 was down regulated in SCA1 cerebella compared to healthy controls and miR-144 also suppresses ataxin-1 expression similarly to miR-19, miR-101 and miR-130 [50]. Furthermore, miR-144 was strongly activated in the aging brains of primates and Alzheimer's patients, which suggests that miR-144 could play a crucial role in post-transcriptional regulation of a unique group of genes that are under selective miRNA control in the brain during aging. A recent study performed miRNA profiling in the cerebella of transgenic SCA1 mice of various ages but could not confirm a major role for miR-19, miR-101, miR-130, or miR-144 in the developing SCA1 mouse neuropathology [51]. It suggested that increased miR-150 levels indirectly modify SCA1 pathogenesis via suppression of *Vegfa* levels in cerebellar neurons, indicating that the outcomes of miRNA profiling studies are tissue- and species-specific. miRNA profiling in the serum of SCA3 patients showed that miR-25, miR-125b, miR-29a and miR-34b could be potential biomarkers for SCA3 [52]. Both miR-25 and miR-125b were predicted to bind to the 3′-UTR of the ataxin-3 gene and may thereby regulate ataxin-3 expression, whereas miR-29a and miR-34b expression was most notably changed in SCA3 serum. miR-29a was already reported to be down-regulated in Alzheimer's disease brains, whereas miR-34b levels were significantly increased in the plasma of presymptomatic Huntington's disease patients [53,54]. In FRDA blood samples, profiling revealed elevated levels of miR-886-3p that correlated with reduced frataxin protein levels, and knockdown of miR-886-3p led to rescue of the suppressed frataxin levels *in vitro* [55]. Whether the actions of miR-886-3p on frataxin are direct or indirect is not known.

Altogether, these studies showed that miRNAs are strong modifiers of the cerebellar ataxia neuropathology and similar regulatory mechanisms may exist for other neurodegenerative disorders. Additionally, mutations in miRNA-binding sites or mutations in miRNAs might underlie the group of genetically undiagnosed cerebellar ataxia patients.

3.3. Methylation profiling

The epigenetic machinery controls many physiological processes, including brain development by regulating gene expression via DNA methylation and deacetlylation/acetylation, and methylation of histones remodeling the chromatin [56]. Epigenetic dysregulation underlies complex neurodegenerative disorders such as Alzheimer's and Parkinson's disease [57,58], but no compelling evidence for this process has been published for the monogenetic cerebellar ataxias. However, very recently, a mutation in the DNA methylation factor, *DNMT1*, leading to global hypomethylation and genomic site-selective hypermethylation, was reported to cause cerebellar ataxia, deafness and narcolepsy syndrome [59]. Additionally, in many repeat expansion disorders, including SCA1, a role for epigenetic alterations in pathogenesis has been proposed [60]. In this case, epigenetic regulation might explain the somatic repeat instability or may play a direct role in the toxic gain of protein function. Moreover, particular DNA methylation profiles of the frataxin, FRDA disease gene, and its locus are not only correlated with mutant frataxin expression, but also with the age of onset and disease severity [61].

Only one study took the opportunity to study the genome-wide transcriptional and epigenetic changes in early onset polyQ-disease in parallel, using the HD-82Q mouse model [62], which could be translational for other repeat expansion disorders, including some of the spinocerebellar ataxias. In this study, many hypoacetylated loci were detected for H3K9,14 and H4K12 in hippocampal chromatin using CHIP-seq, and correlated with only a subset of transcriptional dysregulated genes. However, these dysregulated genes were consistently detected in the different brain areas of a Huntington's disease mouse model and tissue from patients, and are thus likely to play a role in the disease pathology. The functional categories of these genes were significantly enriched for the GO terms synaptic vesicle, synapse, postsynaptic membrane, and calcium ion binding.

Given the limited number of publications, methylation profiling using CHIP-seq clearly still needs to be explored for the insights it can yield into neurodegenerative disorders, including cerebellar ataxias.

4. Genome wide genetic modifier screens in cerebellar ataxia models

Genetic modifiers play an important role in neurodegenerative disorders, influencing the age of onset, progression and severity of the disease. Identifying such modifiers would greatly improve our understanding of the underlying pathology of the disease and could lead to novel therapeutic targets. For ataxia, most modifier screens are performed for polyQ ataxias (SCA 1, 2, 3, 6, 7 and 17), since the polyQ disorders form the largest group within the neurodegenerative field, and includes Huntington's disease, the most common hereditary neurodegenerative disorder. Because these disorders are all caused by a glutamine expansion in the disease gene, they are thought to be linked on a molecular level. Therefore, modifier genes found for one of the polyQ disorders are likely to have a modifying function in one or all of the other polyQ diseases.

For ataxia, a number of modifiers have been identified in *Drosophila melanogaster* [63–69]. The fruit fly is widely used as a model for human disorders; its short generation time and well described genetics make it a powerful tool to study neurodegenerative disorders, since the genetic pathways involved in development and disease are strongly conserved between *Drosophila* and mammals. Furthermore, the flies are easily genetically altered to mimic human disease and to screen for genetic modifiers. Screening for these modifiers is performed by crossing disease-mimicking flies with flies containing P or EP element insertions, followed by screening of the F1 progeny for enhanced or attenuated phenotypes.

Genome-wide modifier screens usually yield a high number of modifier genes, which then have to be identified, categorized and validated. Fernandez-Funez et al. [63] reported performing two modifier

screens for SCA1, and identifying 10 suppressors and 23 enhancers. Some of these modifier genes – functioning in the protein folding/heat shock response or ubiquitin-proteolytic pathways – were already known to modify polyglutamine-induced disease. Others were not previously known to modify polyQ disorders, such as genes involved in oxidative and/or chemical stress, the nuclear pore complex, RNA processing, and transcriptional regulation. Because genome-wide screens are costly and time consuming, and give a relatively low yield, a targeted approach may be more suitable. Furthermore, it is important to validate the present findings in a separate model to demonstrate the legitimacy of the screen.

Latouche et al. [67] crossed a fly model for SCA7 with 36 different fly lines characterized by the enhancement or disruption of genes that could, by their function, modulate the SCA7 phenotype. Two genes previously identified as modifiers of SCA1 (*Ppd3* and *mub*) enhanced the SCA7 phenotype and expression of *mub* in adult neurons significantly reduced lifespan of the SCA7 files [63,67]. Interestingly, reduced expression of ATXN2, the causative gene for SCA2 when mutated, strongly suppressed the SCA7 phenotype. This further emphasizes the need to validate the results of a genetic modifier screen in a second model, such as a relevant cell or animal model, as presented by Park and co-workers [68].

A second screening technique uses RNA interference to knock down a single gene in flies. Model flies are crossed with flies expressing an RNAi construct, which produces a small interfering RNA molecule targeted to a specific gene, followed by screening of the F1 progeny for enhanced or suppressed phenotypes [69]. SCA17 is caused by a polyQ expansion in the human TATA-box-binding protein (hTBP) [70]. Because hTBP is a transcription factor, a microarray analysis was performed to study the resulting dysregulation induced by mutant hTBP [69]. This showed that Q/N-rich transcription factors greatly contribute to the transcriptional dysregulation induced by hTBP80Q. One of these Q/N-rich transcription factors, Su(H), a nuclear component of Notch signaling, was identified as a key candidate, since RNAi knock-down of *Su(H)* enhanced the SCA17 phenotype significantly via a direct interaction with hTBP. Conversely, increased expression of Su(H) could rescue the SCA17 phenotype, indicating that the Su(H) levels control transcription regulation and thereby the SCA17 disease phenotype. This work clearly showed the importance of functional validation of findings from RNAi modifier screens. However, further validation in a more biologically relevant system (*e.g.* mouse) should still be performed.

Park et al. [68] performed a cross-species screen in *Drosophila*, human cells and mice, specifically targeting human kinases in order to identify kinases that can modify ATXN1 toxicity. This is based on previous work in which they showed that phosphorylation of ATXN1 regulates ATXN1 protein stability, and that both factors play a crucial role in the development of SCA1 [71]. In a human cell line, all human kinase and kinase-like genes were targeted with short interfering RNAs (siRNAs) to determine the effect on ATXN1 levels. To validate the findings of this screen, siRNAs that changed ATXN1 levels were transfected individually in ATXN1(82Q) expressing cells yielding 50 siRNAs for 45 genes. In parallel, a genetic screen targeted at kinases identified 49 genes that suppressed ATXN1(82Q) toxicity in a *Drosophila* SCA1 model. Both screens were then combined, and revealed 10 human kinases that diminish ATXN1 levels and ATXN1-induced toxicity. A network analysis showed that the MAPK pathway is highly enriched in both screens, because IGF1R, various MEKs, ERK1 and ERK2 were identified. Reduced activity of the MAPK pathway diminished SCA1 motor dysfunction in flies by modulation of ATXN1 levels. Moreover, ATXN1 levels could also be decreased pharmacologically by PD184352 (a MEK1/2 inhibitor), GW5704 (a RAF1 inhibitor), H89 and Ro-31-8220 (MSK1 inhibitors) in human cells.

The work of Park et al. [64] showed that identification of modifier genes is not enough to make progress in understanding the disease etiology of SCA1. Modifier genes that suppress the disease phenotype should be studied more closely to validate the genes as modifiers, to determine whether the identified genes can be linked via one or two pathways and whether a stellar protein of the pathway can be developed as a drug target.

5. Generation of a cerebellar ataxia network

To get a clearer and more accurate picture of the common disease pathways, we generated a gene–gene interaction network based on

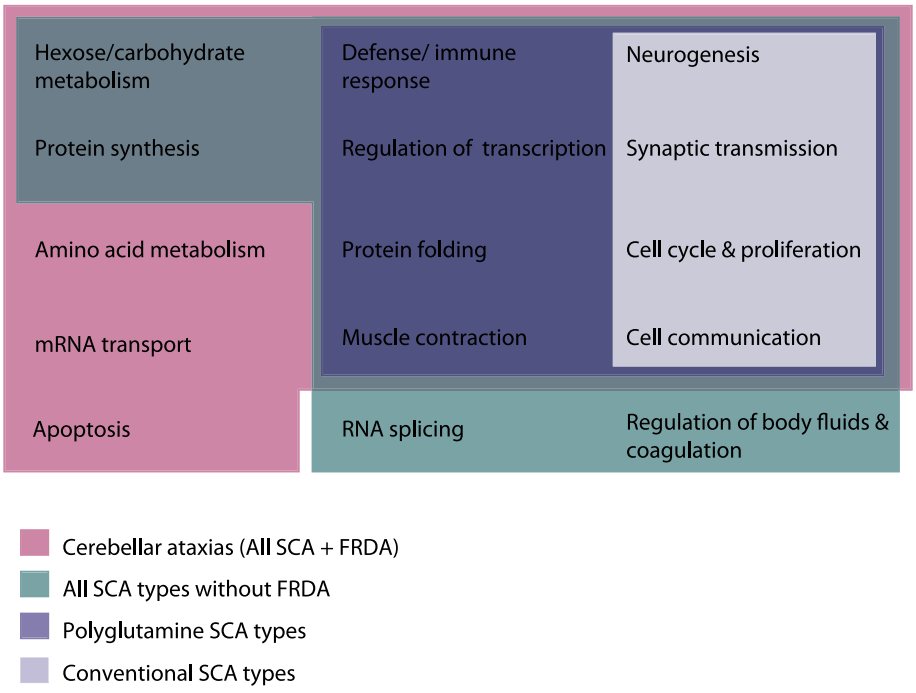

Fig. 1. Diagram illustrating the overlap of pathways identified in gene–gene interaction networks derived from all cerebellar ataxias with or without FRDA, polyglutamine SCA types, and conventional SCA types (see Table 1 for more details).

the co-expression of genes based on a total of approximately 80,000 microarrays from Gene Expression Omnibus (see more at: http://genenetwork.nl:8080/GeneNetwork/#!) with novel cerebellar ataxia genes identified via exome sequencing and significant, previously reported, differentially expressed genes or modifier genes of known cerebellar ataxia genes (see Supplementary Table 1; the software used for generation of the network – GeneNetwork – was written by J. Karjalainen and Dr. L. Franke, UMCG, and is unpublished). When we

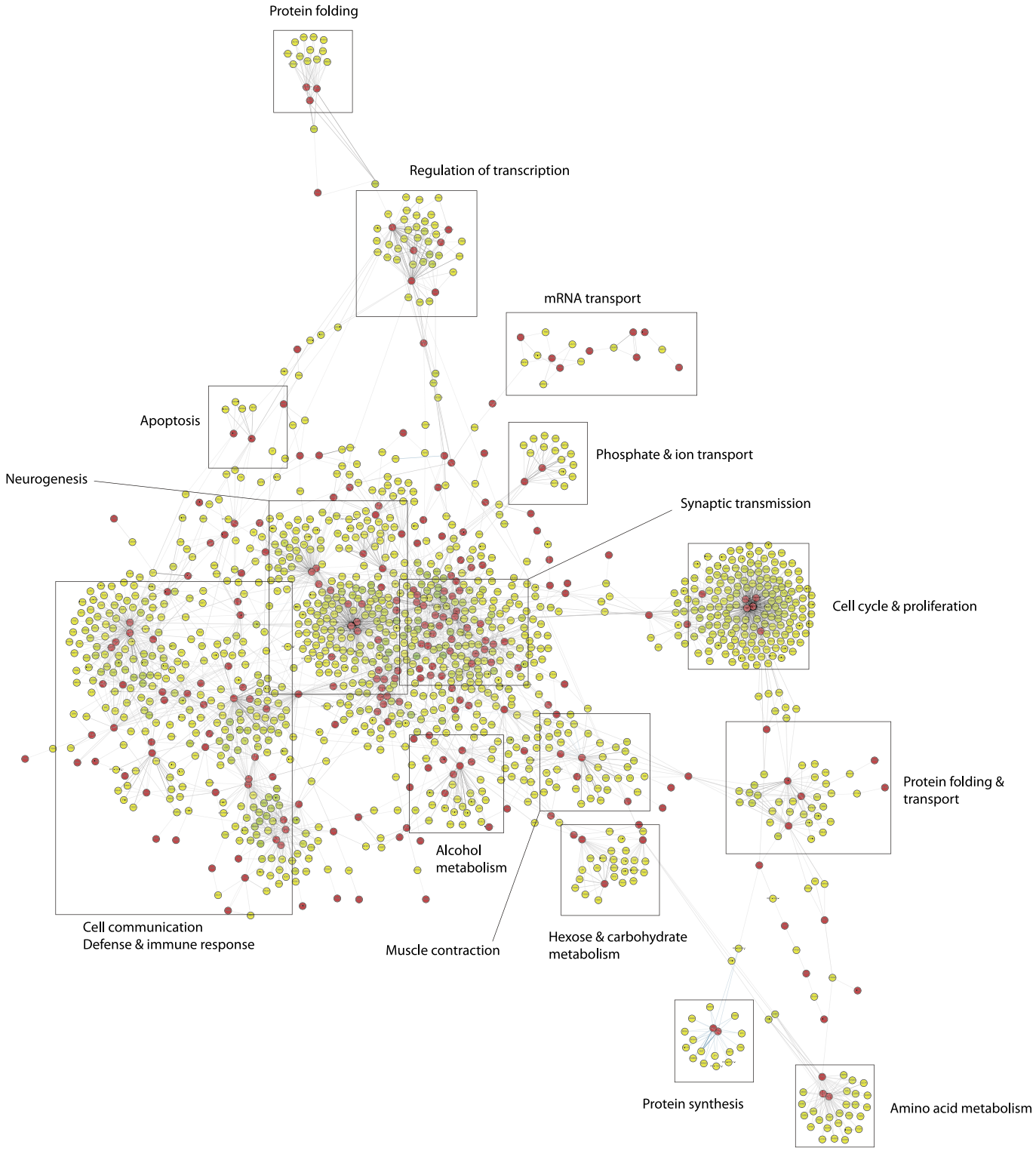

Fig. 2. Cerebellar ataxia gene–gene interaction network including conventional SCA types, polyglutamine SCA types and FRDA (see Table 1 for more details). This network is based on a total of approximately 80,000 microarrays from the Gene Expression Omnibus. The interactions between the genes are based on co-expression. The red genes are the input genes, the yellow-to-green genes, indicating an increasing number of connections, are the co-expressed genes. This network helps to predict gene function and can reveal the underlying pathway. The p-value threshold was set to 1×10^{-10}. The boxes indicate the putative biological pathways identified using the program GATHER by analyzing the gene ontology of the co-expressed genes within the indicated clusters.

Table 2
List of input genes and corresponding predicted pathway using GATHER.

Gene names	Predicted pathway
APP, PDIA3, SEC61A1, CALR	Protein folding & transport
ATF5, CBS, SLC7A1	Amino acid metabolism
DNMT1, GAS2L3, PBK, SPAG5, LMNB1, CKAP5	Cell cycle & proliferation
COL9A3, COL9A2	Phosphate & ion transport
TTC14, SFPQ, PABPNA	mRNA transport
DUSP1, KLF4, NR4A1, RGS2, ADAMTS1	Regulation of transcription
BAG3, HSPA1B, CHORDC1	Protein folding
NINJ1, BIRC3, WDFC2	Apoptosis
MOG, MAG, MOBP, MBP, NINJ2, CDK18, ENPP2, TF, TTR, GJB1, NRGN, C6ORF25, CALB1, KL, PCP2, HOMER3, DYNC1L1, SLC25A18,	Neurogenesis (contains coagulation factors)
HPCA, CBLN2, NGEF, SIX3, KIF5A, SYNPR, CHL1, DRD2, GRM1, PLP1, SLC12A2, CPNE6, RASAl1, ITPKA, RTN4Rm NECAB3, CHL1, NRSN1, AP3B2 SYNGR3, SLC17A6, CHN2, SEZ6, GNG13, KCNIP4, RTN1, NPTXC1, GABRD, PRKCG, PENK, RSG4	Synaptic transmission
LILRA2, CTSS, CSF1R, EMR1, ADORA3, SLA, GAL3ST4, LGMN, PLAG7, CYP27A1, ITGAL, ACP5, APOE, PLD3, ATP2A3, BLK, LEF1, CREG1, NKG7, HS3ST2, PPP2R2B, PRKCH, EOMES, PTPN4, FYN, GIMAP5, GIMAP6, EBF1, EGFL7, RAMP2, ADCY4, EPAS1, ACE, COL18A1, AGRN, PLXDC1, LGR5, FXYD6,	Cell communication & defense/immune response
BCL11A, MCTP2, PIM2, ABCA1 CAP2, MYBPC3, CACNA1C, FHOD3	Muscle contraction
SCARB1, ALDOC, DHCR24, VEGFA, AGRP	Hexose & carbohydrate metabolism

generated a network for only the conventional SCA types (see Table 1), it showed that many of these genes connect directly or indirectly with each other and act in pathways such as neurogenesis, synaptic transmission, cell communication, defense/immune response, and protein folding (Figs. 1 and 2, and Table 2). Many of these pathways are known to be involved in cerebellar ataxia, with the exception of the defense/immune response [6,7,72].

Upon further refining the network by adding the gene list related to the polyQ SCA types to the conventional SCA types (all SCA types without FRDA), more known pathways emerged including regulation of transcription, cell cycle and proliferation, and RNA splicing. Pathways not previously reported also emerged including hexose/carbohydrate metabolism, muscle contraction, and regulation of body fluids and coagulation (Figs. 1 and 2). How these newly identified pathways contribute to the etiology of cerebellar ataxia is still unknown, and we can only speculate about the involvement of the hexose/carbohydrate metabolism and regulation of body fluids and coagulation in cerebellar ataxia. Brain energy metabolism, for instance, might play an important role in activation of glutamate receptors and the induction of excitotoxicity leading to neuronal cell death, or increased mitochondrial hexokinase II activity can prevent cell death in Parkinson's disease-related neuronal cultures [73,74]. Coagulation inhibitors might treat seizures and epilepsy, and various coagulation inhibitors are in the list of drugs in development for neurodegenerative diseases [75,76]. The putative role of muscle in cerebellar ataxia is shown by muscle Q10 deficiency causing familiar cerebellar ataxia and seizures [77]. Additionally, supplements of Q10 improved muscle strength and the ataxia, and reduced the severity of the seizures, and FRDA mice exhibit mitochondrial impairment of their muscles [78]. Upon adding the FRDA-related genes (all cerebellar ataxias; Fig. 2) to the network, two extra pathways emerged: protein synthesis and mRNA transport (Fig. 1 and Table 2). Notably, no marked DNA damage response, mitochondrial fatty acid beta-oxidation, and/or reactive oxygen species was detected, whereas DNA metabolism and steroid and lipid metabolism were detected in an FRDA specific network (data not shown).

Altogether, this network analysis clearly demonstrates that there are common routes to cerebellar neurodegeneration and ataxia, and it highlights novel molecular mechanisms, including muscle contraction and coagulation, as potential candidates for therapeutic intervention.

6. Future prospects

The work summarized in this review revealed a marked increase in the usage of unbiased genome-wide approaches over the last five years to reveal novel disease genes and the underlying molecular mechanisms of cerebellar ataxias. In the absence of high-quality, post-mortem material, appropriate disease models, including patient-derived iPS cells, *Drosophila melanogaster*, and transgenic, knock-in and knock-out mice, have proven to be valuable in understanding the underlying disease mechanisms. Although conventional SCA types currently outnumber the repeat-expansion SCAs, the main focus of research is still on the polyQ-SCA types, including SCA1 and SCA3, yielding a potentially biased view of the underlying biological pathways that lead to cerebellar ataxia. To overcome this bias, major investments are needed in the generation of disease models for conventional SCA types, as these SCA types seem to be caused by a select group of pathways pointing towards deficits in cell communication, proliferation, and synaptic transmission. Different genetic backgrounds can also introduce a bias in the phenotypes of fly and mouse models [79,80], and should always be considered. Additionally, the presence of genetic modifiers is also reflected in the variance in age of onset and disease severity in polyglutamine SCA types including *RAI1* and *CACNA1A* modifying the age of onset of SCA2 [81–83]. Nevertheless, the use of functional genomic approaches, including whole exome sequencing, genome-wide gene expression profiling, miRNA profiling, epigenetic profiling, and genetic modifier screens have already contributed new knowledge and will continue to provide insights that improve our understanding of the pathology of cerebellar ataxia. This all may lead to the identification of targets opening doors for therapeutic intervention that could prevent disease progression in cerebellar ataxia patients.

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