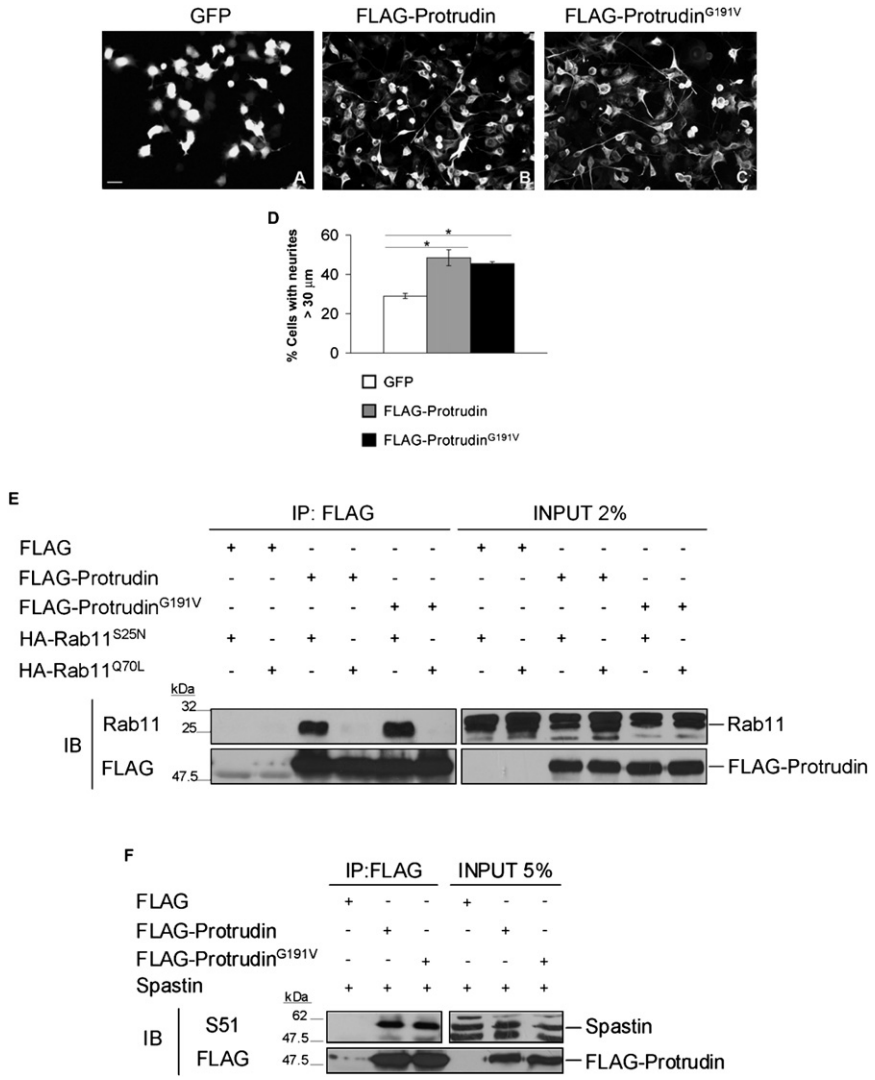

Figure 1. Exploring the Pathogenic Role of Protrudin^{G191V}

(A–C) NSC34 cells were transfected with control GFP, FLAG-protrudin, or FLAG-protrudin^{G191V} vectors. Both wild-type protrudin and protrudin^{G191V} stimulate neurite elongation with the same efficiency. The scale bar represents 50 µm.

(D) Quantification of the percentage of cells with neurites longer than 30 µm in the different conditions (means of at least three independent experiments ± SEM; at least 450 cells were scored per condition; *$p < 0.05$, Student's t test). The average length of neurites in these cells was 58.01 µm ± 3.56 for wild-type protrudin and 59.55 µm ± 3.66 for protrudin^{G191V}.

(E) Coimmunoprecipitation experiments between protrudin and Rab11 show that both wild-type protrudin and protrudin^{G191V} interact with Rab11^{S25N} (GDP-bound form) but not with Rab11^{Q70L} (GTP-bound form).

(F) Spastin interacts both with wild-type protrudin and protrudin^{G191V}. To detect spastin, we used a specific antibody (S51).

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

Single Nucleotide Polymorphism (SNP) database, http://www.ncbi.nlm.nih.gov/projects/SNP/

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Response to Martignoni et al.

To the Editor: I appreciate this opportunity to respond to the letter by Martignoni et al. In the letter, Martignoni et al. raised certain concerns over our previous finding, where we showed that ZFYVE27 (MIM 610243) interacts with spastin and is mutated in a German family with an autosomal-dominant form of hereditary spastic paraplegia (HSP).[1] The index patient of this HSP family was screened for mutations in the *SPG4* gene (MIM 604277) and was negative for any mutation.

We identified ZFYVE27 as spastin-interacting protein in a yeast two-hybrid screen, then went on to validate the interaction between spastin and ZFYVE27 in mammalian cells by coimmunoprecipitation and colocalization

studies.[1] Furthermore, we showed that endogenous spastin could interact with overexpressed ZFYVE27. Martignoni et al. were also able to show that ZFYVE27 is a spastin-interacting protein, which serves as an independent validation of this interaction. In our study, we observed a diminished interaction between mutated ZFYVE27 (p.G191V) and spastin;[1] however Martignoni et al. reported that both wild-type and mutated ZFYVE27 interact with spastin with similar ability. This discrepancy between our observation and that of Martignoni et al. could be due to the different systems of overexpression assay used for detecting this interaction. Moreover, from Figure 1F of Martignoni et al., it appears that there is a very high level of overexpression of mutated ZFYVE27, which might mask a subtle effect on its interaction with spastin.

In a recent study, Shirane and Nakayama showed that overexpression of ZFYVE27 in HeLa cells promotes neurite extension in 5% to 30% of transfected cells.[2] Martignoni et al. did not observe any difference in the ability of wild-type and mutated ZFYVE27 to promote neurite outgrowth. We have also performed a similar study; however, we observed a much more pronounced effect of GFP-ZFYVE27^{G191V} than of wild-type GFP-ZFYVE27 on the promotion of neurite outgrowth (Figure 1). Furthermore, in silico analysis of the primary sequence of ZFYVE27 revealed a TGN-endosome sorting motif ^{186}YGAL^{189} (YXXΦ), which is abolished as a result of the p.G191V mutation.

Martignoni et al. brought to our attention that the sequence variant c.572G > T (p.G191V) in *ZFYVE27*, which we identified in the German HSP family, is also present in several control populations from different ethnic backgrounds. At the time of our publication, this sequence variant was not published in the SNP database. Nevertheless, the possibility of a variable level of pathogenic and nonpathogenic effects of the p.G191V sequence variant in different ethnic populations cannot be excluded from this observation. In several genetic disorders, it is been reported that an identified sequence variant is either pathogenic or nonpathogenic in different racial backgrounds. A well-characterized sequence variant is p.M34T in *GJB2* (MIM 121011); mutation in this gene causes congenital hereditary nonsyndromic sensorineural deafness. The p.M34T was originally reported as a pathogenic mutation with a dominant effect. Later, these findings were questioned, and p.M34T was suggested to act in a recessive, hypomorphic, or nonpathogenic allele in different ethnic populations (summarized in Pollak et al. 2007).[3] When the role of M34T was reassessed by biochemical and electrophysiological studies, it was concluded that this mutation was pathogenic and caused mild hearing impairment.[4] However, we agree with Martignoni et al. that the pathogenic effect of the p.G191V sequence variant detected in ZFYVE27 in one HSP family (so far) should be interpreted with caution until additional causative sequence variant(s) are identified in *ZFYVE27* in further HSP cases.

To date, in addition to ZFYVE27, several spastin-interacting proteins have been identified, namely; RTN1, atlastin, CHMP1B, and NA14. However, to our knowledge, the functional significance of any of these interacting proteins (apart from ZFYVE27) with spastin in a cellular pathway relevant to HSP has not yet elucidated. Conversely, the role of ZFYVE27 and spastin in a molecular process relevant to HSP is highlighted by recent functional studies on ZFYVE27 and spastin in neurons.[2,5] Recently, Shirane and Nakayama illustrated that ZFYVE27 (protrudin) plays a central role in membrane trafficking in neurons and promotes neurite extension.[2] They speculate that protrudin and spastin might together constitute a system for the regulation of vesicular transport in neurons. Moreover, in a latest publication, Yu et al. showed that overexpression of spastin in primary hippocampal neurons leads to extensive neurite outgrowth similar to that observed for protrudin.[5] Together, these findings strengthen the role of spastin and ZFYVE27 (protrudin) in a common cellular pathway vital for neurons.

Finally, the role of the FYVE family of proteins in HSP pathogenesis is further reinforced by identification of mutations in *ZFYVE26* (MIM 612012) in the SPG15 subtype of HSP.[6]

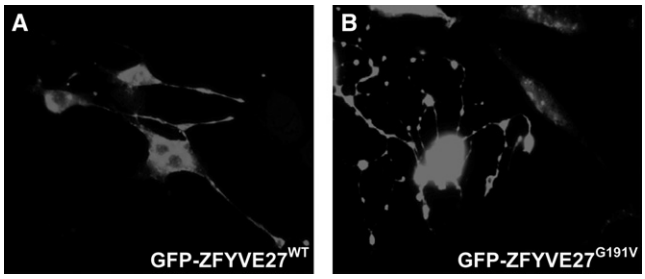

Figure 1. Overexpression of GFP-ZFYVE27^{WT} and GFP-ZFYVE27^{G191V} in NIH 3T3 Cells

When GFP-ZFYVE27^{WT} was overexpressed, we observed a moderate level of neurite extension in the cells that had been transfected with GFP-ZFYVE27^{WT} (A). However, overexpression of GFP-ZFYVE27^{G191V} led to a pronounced outgrowth of neurites from a distinctive cell soma (B).

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Web Resources

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *SPG4*, *ZFYVE26*, *ZFYVE27*, and *GJB2*).

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Personalized Genetics: A Responsible Approach

To the Editor: A recent paper[1] in *The Journal* aptly described the challenges inherent in using genomic profiles to predict risk for common diseases and to develop personalized risk-prevention advice. Companies or other organizations that take a responsible approach to these challenges can potentially offer new opportunities for disease prevention, early detection, and treatment.

The authors took a sample of the loci covered by the tests of seven companies in the field and have shown that most of these loci do not pass simple quality criteria. The challenge with the authors' analysis is that it analyzes the pool of loci used by all seven companies, instead of breaking the analysis down by company or organization; hence, the approach does not distinguish between organizations that take a rigorous and responsible approach to the evaluation of risk and organizations that base the risk assessment on unreliable scientific information.

We share the authors' concerns about companies that report genetic risk based on a single association study or on studies with methodological weaknesses. However, we strongly believe that customers can benefit from a personalized report of those genetic associations found in genome-wide-association studies that were replicated in multiple populations with sound epidemiological, statistical, and laboratory practices. Many examples of reliable, replicated associations have been reported, including between transcription factor TCF7L2 (MIM 602228) and diabetes and between NOD2 (MIM 605956) and Crohn's disease.[2]

Taking a responsible approach means that companies utilize only high-quality association studies to bring customers accurate genetic risk predictions, as well as effective strategies for reducing risk for those genetic conditions to which they are predisposed. In addition to using rigorous and transparent scientific standards for inclusion in the testing panel, a responsible approach is to provide customers and their doctors with resources such as genetic counselors, physician expertise, and epidemiologists. By taking a responsible approach, the personalized genomics community can work together with individuals and their medical providers to enable people to live longer, healthier lives.

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Web Resources

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