EBioMedicine 7 (2016) 21–22

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Commentary

Neurofibromatosis Type 1 Molecular Diagnosis: The RNA Point of View

Eric Pasmant *, Dominique Vidaud

Department of Molecular Genetics and Biology, Cochin Hospital, Hôpitaux Universitaires Paris Centre, AP-HP, and EA7331, Université Paris Descartes, Sorbonne Paris Cité, Faculté de Pharmacie, Paris, France

ARTICLE INFO

Article history:
Received 26 April 2016
Accepted 26 April 2016
Available online 30 April 2016

Keywords:
NF1
SPRED1
RNA

Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders and is caused by mutations in the *NF1* (*Neurofibromin 1*) gene. More than half of all NF1 cases are caused by *de novo* sporadic mutations. Mutation detection is challenging owing to the large size of the *NF1* gene, the presence of numerous pseudogenes, and the great variety of possible alterations. Recurrent *NF1* locus large deletions (found in 5 to 10% of NF1 patients) have been associated with more severe and atypical manifestations (Pasmant et al., 2010).

In this issue of *EBioMedicine*, Evans and colleagues report their experience with a RNA-based *NF1* gene mutation analysis in the English NF1 reference laboratory between 2009 and 2015 (Evans et al., 2016). A comprehensive cDNA analysis coupled with multiplex ligation-dependent probe amplification (MLPA) at DNA level (for large deletions detection) was performed in a cohort of 361 English NF1 patients.

The aim of the study was to present a molecular strategy for *NF1* alteration detection focused on a cDNA-targeted approach by Sanger sequencing. This cDNA-based approach was previously modelled (Messiaen et al., 2000; Pros et al., 2008; Valero et al., 2011; Sabbagh et al., 2013). However, this study by Evans and colleagues provides important information on the effectiveness of screening a large cohort of NF1 patients. Moreover, they provide a comprehensive list of annotated mutations. Interpretation of *NF1* variants is challenging (in particular for missense and in frame variations): a huge number of different pathogenic mutations have been reported in the tumour suppressor gene *NF1*. It appears that a significant proportion of *NF1* missense mutations is deleterious by affecting normal pre-mRNA splicing. The characterization of mutations at cDNA level thus enables the detection of a broader spectrum of mutations, and provides a greater understanding of their molecular pathogenesis (Messiaen et al., 2000; Pros et al., 2008; Valero et al., 2011). The mutation collection provided by Evans and colleagues is therefore very useful for molecular diagnosis, in particular because the functional effects of variation were assessed at mRNA level.

Evans and colleagues also underlined that sequencing at the cDNA level can increase mutation detection sensitivity due to the presence of deep intronic mutations, or exons deletions (Imbard et al., 2015). Their screen for *NF1* gene lesions identified ~96% of pathological mutations in patients presenting with typical NF1, using a two-step approach including a cDNA Sanger sequencing and copy number variations (CNV) study. This high mutation detection sensitivity can be achieved in well phenotyped NF1 patients. It is comparable to the one obtained with previous screening methodologies, confirming the interest of this mRNA-based approach (Messiaen et al., 2000; Pros et al., 2008; Valero et al., 2011; Sabbagh et al., 2013; Pasmant et al., 2015). In the study by Evans and colleagues, RNA was isolated from short-term phytohemagglutinin (PHA) cultures pre-treated before RNA extraction with Puromycin to inhibit nonsense mediated decay. Short term PHA culture allows better control of the cell culture conditions and avoidance of spurious alternative splicing and mRNA decay. However, in our experience, an approach using PAXgene™ Blood RNA System (Qiagen) may simplify and shorten the process, allowing stabilization of RNA at the time of sample collection, and avoiding artefacts introduced during sample handling (Sabbagh et al., 2013).

A correct NF1 diagnosis has important implications for prognosis, counseling, and prenatal diagnosis. Molecular diagnosis in NF1 is helpful to confirm the clinical diagnosis, notably in patients with paucisymptomatic, pediatric, or segmental presentations. In young children, café-au-lait macules are often the only clinical findings. In these cases, mutation analysis of the *NF1* gene may clarify the diagnosis if *de novo*. Clinical applications of mutational analysis have increased in relevance since some clear genotype-phenotype correlations have been identified (in patients with large *NF1* locus deletions, missense mutations affecting codon p.Arg1809, or inframe deletions of codon p.Met992) and because of clinical overlap with the Legius syndrome (Pasmant et al., 2012). Limited genetic heterogeneity has been found in NF1 by identification of the Legius syndrome, caused by mutations in the *SPRED1* gene encoding a negative regulator of the RAS-MAPK pathway (Brems et al., 2012). *SPRED1* mutations are rare compared to *NF1* mutations. Patients with Legius syndrome have multiple café-au-

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2016.04.005.
* Corresponding author at: Department of Molecular Genetics and Biology, Cochin Hospital, 27 rue du Faubourg Saint Jacques, Paris, France.
E-mail address: eric.pasmant@parisdescartes.fr (E. Pasmant).

http://dx.doi.org/10.1016/j.ebiom.2016.04.036

lait spots and/or skinfold freckling but do not develop the typical NF1-associated tumours. Because of the important clinical overlap with NF1, it is impossible to diagnose the syndrome exclusively based on clinical feature alone. In patients presenting solely with café-au-lait spots and/or frecklings, a molecular confirmation has to be performed. Molecular diagnosis is essential because distinguishing between NF1 and Legius syndrome is important for prognosis and clinical management.

Evans and colleagues also report six variants in an evolutionary conserved region of the *NF1* 5′ untranslated region (UTR), with bi-allelic *NF1* expression and a *de novo* occurrence in one case. This interesting finding suggests a previously unreported mechanism of *NF1* loss-of-function that needs further experimentation. Finally, for the remaining ~4% of NF1 patients with no molecular confirmation after *NF1* and *SPRED1* screening, a whole exome sequencing approach could be used to search for additional NF1 phenotype disease loci.

References

Brems, H., Pasmant, E., Van Minkelen, R., et al., 2012. Review and update of SPRED1 mutations causing Legius syndrome. Hum. Mutat. 33, 1538–1546.

Evans, D.G., Bowers, N., Burkitt-Wright, E., et al., 2016. Comprehensive RNA analysis of the NF1 gene in classically affected NF1 affected individuals meeting NIH criteria has high sensitivity and mutation negative testing is reassuring in isolated cases with pigmentary features only. EBioMed. 7, 212–220.

Imbard, A., Pasmant, E., Sabbagh, A., et al., 2015. NF1 single and multi-exons copy number variations in neurofibromatosis type 1. J. Hum. Genet. 60, 221–224.

Messiaen, L.M., Callens, T., Mortier, G., et al., 2000. Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. Hum. Mutat. 15, 541–555.

Pasmant, E., Sabbagh, A., Spurlock, G., et al., 2010. NF1 microdeletions in neurofibromatosis type 1: from genotype to phenotype. Hum. Mutat. 31, E1506–E1518.

Pasmant, E., Parfait, B., Luscan, A., et al., 2015. Neurofibromatosis type 1 molecular diagnosis: what can NGS do for you when you have a large gene with loss of function mutations? Eur. J. Hum. Genet. 23, 596–601.

Pros, E., Gómez, C., Martín, T., et al., 2008. Nature and mRNA effect of 282 different NF1 point mutations: focus on splicing alterations. Hum. Mutat. 29, E173–E193.

Sabbagh, A., Pasmant, E., Imbard, A., et al., 2013. NF1 molecular characterization and neurofibromatosis type I genotype-phenotype correlation: the French experience. Hum. Mutat. 34, 1510–1518.

Valero, M.C., Martín, Y., Hernández-Imaz, E., et al., 2011. A highly sensitive genetic protocol to detect NF1 mutations. J. Mol. Diagn. 13, 113.