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A newly implemented NGS-based methods to detect GBA variants in patients with Parkinson's disease

Edoardo Monfrini[1], I. Palmieri[2], G. Cuconato[3], M. Percetti[1], M. Morelli[4], E. Zapparoli[4], A.B. Di Fonzo[1], E.M. Valente[2,3]

[1]IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[2]Neurogenetics Research Center, IRCCS Mondino Foundation, Pavia, Italy
[3]Dept. of Molecular Medicine, University of Pavia, Pavia, Italy
[4]IRCCS San Raffaele, Milan, Italy

Introduction: Heterozygous variants in the *GBA* gene, encoding for the lysosomal enzyme β-glucocerebrosidase, are the most common genetic risk factor for Parkinson disease (PD), accounting for 5-15% of all PD cases. Sequencing of the whole *GBA* coding region (11 exons) is a burdensome task, both employing conventional techniques such as Sanger sequencing as well as more innovative strategies such as next-generation-sequencing (NGS). In particular, the high degree of homology (96-98%) between *GBA* and its pseudogene *GBAP* often leads to recombination events that eventually produce complex alleles which are misaligned and missed by the standard NGS pipeline.

Objectives: We implemented a NGS-based technology on a selected pool of 100 PD patients, including negative and positive controls.

Methods: The NGS experiment was designed to start from a specific long-range PCR which amplifies a unique 6 kb amplicon encompassing the *GBA* gene only. This was used as template to create libraries, which were amplified using Nextera technology and then run on an Illumina MiSeq instrument. In parallel to standard bioinformatic analysis, a tailored pipeline was used, masking *GBAP* pseudogene on the reference sequence and forcing the alignment of reads against the *GBA* gene only.

Results: All known *GBA* variants were correctly called and identified using this approach; furthermore, comparing (*.bam) files obtained with standard vs forced alignment, the latter showed a significant increase in read depth and mapping quality.

Conclusion: The proposed NGS-based approach appears a reliable and valid alternative for *GBA* sequencing, holding promise to increase speed analysis and variant detection rate compared to conventional strategies.