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NGS in neurodegenerative disorders - our experience

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Next Generation of Sequencing-NGS

- Sanger sequencing - longer reads, more accuracy, but slow and expensive
- NGS - parallelization of high number of sequencing reactions
- Whole Genome Sequencing (WGS), Whole Exome Sequencing (WES), gene panels („clinical exome“)

„Clinical exome“

- Coding regions ~ 5000 genes which are related to known clinical phenotypes (TruSight One Illumina panel)
- Advantages (comparing to WES and WGS):
- 1) lower price
- 2) easier data analysis
- 3) better coverage
- 4) Illumina MiSeq platform

General strategy for NGS aplication in our lab

- Analysis of genes related to neurodegenerative disorders characterized by the development of motor impairments (parkinsonism, dystonia, ataxia, tremor) and cognitive impairments
- Selection of subjects to be tested:
- Ahead of the study, standard DNA tests had been used to exclude patients with possible genetic disorders that could lead to the corresponding phenotype

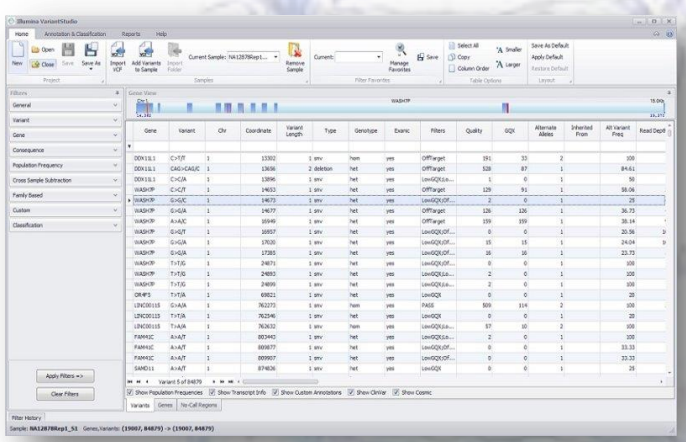
General strategy for NGS aplication in our lab

- Preference was given to family cases where further comparative analysis of the proband and his/her healthy/ill relatives was possible,
- Early onset of disease,
- Phenotype with genetic heterogeneity,
- Phenotype which did not correspond to any typical clinical forms of neurodegenerative pathology (i.e. having certain features or cobining several syndromes)
- Phenotype that implied specific genes, which are either rather long for routine diagnostics or rare (i.e. with undeveloped molecular-genetic research protocols)

Library preparation and data analysis

- Technical suport - Elta90MS
- Library preparation – DNA from blood sample
- Library quantification - real time, qubit, bioanalyzer

- Illumina Variant Studio v3- user friendly software



Patients

- Case 1: A male patient with gradually progressing ataxia, oculomotor impairments (oculomotor apraxia). As the result of the NGS analysis, we identified a pathogenic missense mutation I1942T in the exon 14 of the *SETX* gene in a homozygous state. This mutation is pathological and associated with the development of autosomal recessive disease – ataxia with oculomotor apraxia type 2 (AOA2).
- Case 2: A 62-year old male patient who has suffered from dystonia for 15 years, having a novel missense mutation L239P in the exon 6 of the *PDGFRB* gene in a heterozygous state. According to *in silico* prediction softwares, this mutation is pathogenic, and associated with the autosomal dominant Fahr's syndrome.

Patients

- Case 3: A 40-year old female patient who has suffered from dystonia since she was 9, with the family history of movement impairment.
- Still unsolved case
- Further analysis

Case No.	Clinical picture	Gene	Mutation	Zygosity	Diagnosis	Inheritance type
1	Ataxia, apraxia	*SETX*	I1942T	Hom	AOA2	AR
2	Dystonia	*PDGFRB*	L239P	Het	Fahr's syndrom	AD
3	Dystonia	NA	NA	Het	NA	AD

Het: heterozygous mutation, Hom: homozygous mutation, AD: autosomal dominant, AR: autosomal recessive; NA- causative mutation not identified

Current and future applications of NGS in neurological disorders

- NGS technologies provide new opportunities in helping the diagnosis of neurological disorders characterized by high genetic and phenotypic heterogeneity
- Besides having several genes linked to a group of similar disorders, different mutations in one gene can lead to a broad phenotype spectrum of neurological disorders demonstrating that shared neurological pathways may underlie clinically distinct phenotypes
- Finding treatments, cures and methods for the early diagnosis of neurological diseases is a goal of increasing urgency and recent advances in the uses of NGS technologies in genomic medicine provide new opportunities towards this goal

Conclusion

- We have successfully run 30 patients (10 runs) for a year and a half
- Achievments: more successful diagnosis, large amount of data
- Future – standard genetic test in our laboratory

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