Next Generation Sequencing as a good standard for diagnosis of mitochondrial disorders

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Introduction: Mitochondrial diseases are a group of clinically and genetically heterogeneous disorders characterized by variable penetrance and expressivity, with a different age of onset.

Most of the disease-causing mtDNA mutations are heteroplasmic and the degree of heteroplasmy can vary in different tissues. Indeed notable diseases as myoclonic epilepsy with ragged red fibers (MERRF) and myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) are caused by mutations in the tRNA genes MTTK (mitochondrially encoded tRNA lysine) and MTTL1 (mitochondrially encoded tRNA leucine 1), respectively, and there is often substantial overlap in the clinical presentation of the two syndromes.Thus, the determination of mutant loads in affected tissues is important for the diagnosis in order to correlate the clinical phenotype.

Next Generation System (NGS) approach allows simultaneous analyses of a group of genes or of the whole exome, thus, the mutations in causative gene(s) can be identified in one-step. Our purpose was to perform genetic analyses in a faster and more efficient way and to identify the specific degree of heteroplasmy.

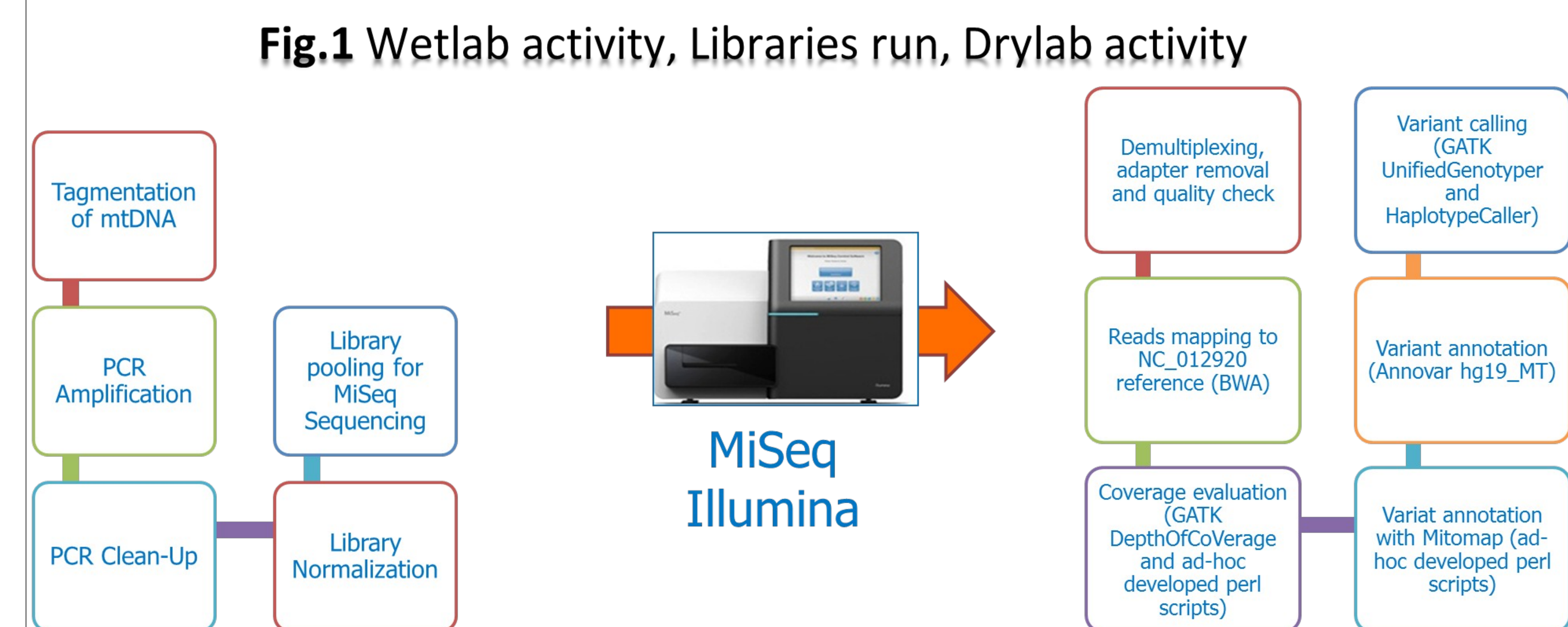

Fig.1 Wetlab activity, Libraries run, Drylab activity

Table 1: results of NGS analisys

Sample ID	MtDNA Haplogroup	Haplotype[a]
173-11	H2a2b	263 309.1C 315.1C 5515 8344R (87.6%) 8860 9759 15326 16235 16291 16519
112-14	J1c17	73 185 228 263 295 315.1C 462 489 750 1438 2140 2706 3010 3847 4216 4769 7028 8860 10398 11251 11719 12612 12634 13708 14766 14798 15326 15452A 16069 16311 16519
85-15	J1c3	73 185 263 295 315.1C 462 489 750 1438 2706 3010 4216 4769 5393 7028 7080 8860 8920 9171 10398 11251 11719 12612 13708 13934 14178 14766 14798 15326 15452A 16069 16093 16126 16189
83-14	T2c1e	73 146 263 309.1C 315.1C 523-524d 709 750 1438 1888 2706 4216 4769 4917 6261 7028 8697 8860 10289 10463 10822 11251 11719 11812 13368 13889 14233 14766 14905 15326 15452A 15607 15928 16126 16129 16292 16294 16296 16380 16519
169-15	U3a2a1	73 150 189 200 263 310Y(19%)C 315.1C 750 1438 1811 2294 2706 4703 4769 6050 6518 7028 8737 (ATP6 M71V) 8860 9266 10143 10506 11050 11467 11719 12308 12372 13934 14139 14766 15326 15454 16343 16390 16519

Materials and Methods: A cohort of 52 Italian patients with Mitochondrial Disease was analyzed. DNA was extracted from Peripheral Blood Mononuclear Cells (PBMC) and mtDNA libraries have been prepared by NexteraXT Platform kit (Illumina). Data collected from NGS experiments were analyzed in order to identify single nucleotide variants and small insertions/deletions (Fig.1). Sanger sequencing was used for variant confermation in the target genes.

Results: Data analysis confirmed the classical mutation 8344R in a sample used as a positive control and showed the presence of risk factors as 7080, 13889 and 12634. Data anlysis show the precence of new mutation A8737G (ATP6 M71V) not previusly reported in one sample (Tab. 1). The most interesting data concerned a sample which, with the restriction fragment length polymorphism (RFLP) methodology, did not show the presence of the 3243R mutation which, on the other hand, was observed at 13.6% with NGS technology (Fig.2).

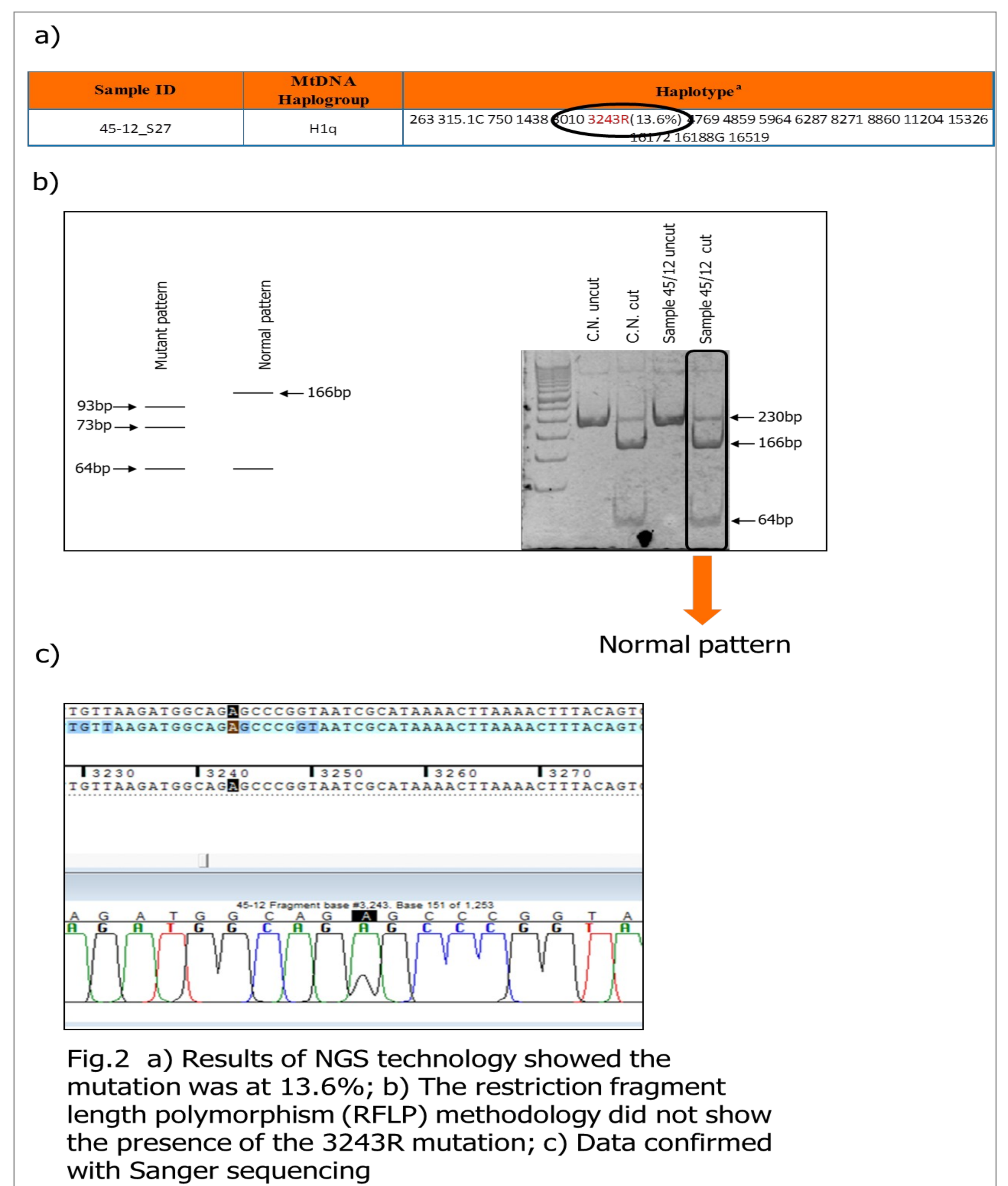

Fig.2 a) Results of NGS technology showed the mutation was at 13.6%; b) The restriction fragment length polymorphism (RFLP) methodology did not show the presence of the 3243R mutation; c) Data confirmed with Sanger sequencing

Discussion: Our results confirmed that the NGS approach is a more sensitive and specific technique compare to other methods to study the mtDNA, allowing the simultaneous analysis of a group of genes or of the whole exome. Moreover, the use of deep sequencing allowed the identification of a low-level of heteroplasmy as well as the presence of rare mutations. Functional studies will be performed to investigate pathogenic mechanisms of the missense mutations.

Cardaioli E. et al J.Neurol. 2000; J. L. King et al. Forensic Sci Int Genetics 2014, C. Davis, et al. Legal Medicine 2015

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