Genetic basis of Oculopharyngeal Muscular Dystrophy: Detection of Alanine repeats in *PABPN1* gene by Next Generation Sequencing

Babi Ramesh Reddy Nallamilli, Andrew McCarty, Akanchha Kesari, Yang Wang, Christin Collins, Ruby Liu, Allison Mitchell, Abhinav Mathur, Lora Bean and Madhuri Hegde
PerkinElmer Genomics, Atlanta, GA

INTRODUCTION

Oculopharyngeal muscular dystrophy (OPMD) is characterized by ptosis, dysphagia and selective involvement of the muscles of the eyelids and pharynx. The age of onset of OPMD is usually in the fourth to sixth decade of life. Expansion of a GCN trinucleotide repeat in exon 1 of the *PABPN1* gene is associated with OPMD. It is inherited in either an autosomal dominant or an autosomal recessive manner. Normal alleles have ten GCN repeats encoding ten alanine amino acids. Autosomal dominant alleles have 12 to 17 GCN repeats and autosomal recessive alleles have 11 GCN repeats. Autosomal recessive form of OPMD is characterized by later age of onset along with milder clinical features. It is difficult to understand the genetic basis and complex inheritance mechanisms of OPMD due to rare nature of this disorder (Table 1). Also, with this given complex inheritance mechanism, it is very important to understand the genotype-phenotype correlations.

Table 1. Complex inheritance mechanisms of Oculopharyngeal Muscular Dystrophy

GCN Repeat Alleles	Mode of Inheritance	Predicted Phenotype
10 + 10		Unaffected
10 + 11	Autosomal recessive	Carrier, unaffected
11 + 11	Autosomal recessive	Affected
10 + 12 to 17	**Autosomal dominant**	**Affected**

MATERIALS AND METHODS

Sequencing was performed on genomic DNA using an Agilent targeted sequence capture method to enrich for the genes on this LGMD panel including *PABPN1* gene. Direct

sequencing of the amplified captured regions was performed using 2X100bp reads on Illumina next generation sequencing (NGS) systems. A base is considered to have sufficient coverage at 20X and an exon is considered fully covered if all coding bases plus three nucleotides of flanking sequence on either side are covered at 20X or more. Alignment to the human reference genome (hg19) was performed and annotated variants are identified in the targeted region. Variants were called at a minimum coverage of 8X and an alternate allele frequency of 20% or higher. Sequence variants were assessed by our proprietary analysis and interpretation pipeline, ODIN.

RESULTS

We have developed a comprehensive next-generation sequencing (NGS) based LGMD panel testing in Lantern project. Based on the suggestions from different neurologists across the united states we have included *PABPN1* gene in the LGMD panel testing to cover the overlapping other myopathies like OPMD. To date 5340 samples have been received for this panel testing. Definitive molecular diagnosis was established successful in total of 73 (1.3%) individuals by identifying pathogenic GCN repeat allele in *PABPN1* gene using this NGS based panel testing. Carrier status was also established in total of 30 (0.5%) individuals by identifying heterozygous 11 GCN repeat allele (Table 2).

Table 2. Summary of GCN repeat alleles identified in this study

GCN Repeat Allele	Zygosity	No of cases	Inheritance	Phenotype
11 GCN Repeat Allele	Heterozygous	30	AR	Carrier
11 GCN Repeat Allele	Homozygous	1	AR	Affected
12 GCN Repeat Allele	Heterozygous	3	AD	Affected
12 GCN Repeat Allele	Homozygous	1	**AD**	Affected
13 GCN Repeat Allele	Heterozygous	60	AD	Affected
14 GCN Repeat Allele	Heterozygous	4	AD	Affected
15 GCN Repeat Allele	Heterozygous	4	AD	Affected

To the best of our knowledge, this is the first large scale report of diagnostic testing of the PABPN1 gene using NGS based testing. We have observed different interesting combinations of autosomal dominant alleles and autosomal recessive alleles in this study. The 13 GCN repeat allele was identified in 60 (80%) individuals out of the total 73 definitive diagnostic OPMD cases, indicating it is the most common autosomal dominant pathogenic allele. Homozygous 11 GCN repeat allele was identified in only one individual (1.3%) indicating autosomal recessive OPMD subtype is very rare when compared to autosomal dominant subtype. Other dominant alleles including 12 GCN allele, 14 GCN allele and 15 GCN allele were identified in 12 (16%) individuals indicating these dominant alleles are rare compared to 13 GCN allele. It is interesting to note that homozygous 12 GCN repeat allele was identified in one individual with OPMD.

CONCLUSION

- The 13 GCN repeat allele was identified in 60 (80%) individuals out of the total 73 definitive diagnostic OPMD cases, indicating it is the most common autosomal dominant pathogenic allele.
- Homozygous 11 GCN repeat allele was identified in only one individual (1.3%) indicating autosomal recessive OPMD subtype is very rare when compared to autosomal dominant subtype.
- This large scale definitive molecular diagnostic results are very helpful to conduct further genotype-phenotype correlation studies specifically to observe correlation between alanine repeat length with age of onset, disease severity and progression.