The EuroBiotech Journal
MINI REVIEW

MAGI Balkans, a laboratory for the diagnosis of rare genetic diseases

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Abstract

Molecular diagnosis relieves patients of uncertainty, aids informed decisions about health and reproductive choices, and helps them join clinical trials or access available therapy. Genetic testing by next generation sequencing (NGS) is the suggested choice for a wide variety of disorders with heterogeneous phenotypes, alleles and loci. The development of a NGS service at MAGI Balkans, through the support of a partner, increases the availability of forefront genetic testing in Albania with great advantages for patients and their families.

Here we report the NGS tests performed in collaboration with MAGI Euregio, Italy, for the diagnosis of rare genetic disease in seven probands and their families. The diseases/manifestations included ichthyosis, familial adenomatous polyposis, diabetes, syndromic craniosynostosis, fronto-temporal dementia, fragile X syndrome and ataxia. We obtained an overall detection rate of 57%. For 4/7 probands we identified a pathogenic or likely pathogenic variant, while for the others, the results did not completely explain the phenotype. All variants were confirmed by Sanger sequencing. Segregation of the variant with the affected phenotype was also evaluated.

Keywords: craniosynostosis, familial adenomatous polyposis, frontotemporal dementia, Albania, Tirana

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Published online: 28 December 2017
doi:10.24190/ISSN2564-615X/2017/S2.04

Introduction

According to the US National Institutes of Health, Office of Rare Disease Research (ORDR) and Orphanet (http://www.orpha.net), there are approximately 7000 rare genetic diseases (1). Rare diseases are defined as those affecting less than one in every 2000 persons. It is difficult to obtain scientific information and to design clinical research for rare diseases mainly because of the lack of a statistical sample of affected individuals and families. The scarcity of scientific knowledge often means that diagnosis is made long after onset of the disease. Patients with rare genetic diseases typically endure about 6 years of investigations, and in 40% of cases the first diagnosis turns out to be wrong. This delay adversely affects prognosis, resulting in physical, psychological and intellectual damage, despite the fact that a normal life is possible with early diagnosis and treatment in certain cases. A definitive genetic diagnosis can obviate the need for further diagnostic investigations, leading to savings for the health system. It also enables access to healthcare resources, recurrence-risk counseling, informed reproductive decisions in affected families and an end to prognostic uncertainty (2). Genetic diagnosis of rare genetic diseases can enable highly effective targeted therapies in some cases or participation in clinical trials requiring molecular and phenotypic diagnosis (3,4).

MAGI Balkans is a laboratory of biochemical analysis, inaugurated in 2014 to remedy the chronic shortage of blood in Albania, to improve the quality and safety of donated blood, to reduce the practice of paid blood donations in Albania, and to help donors maintain a healthy lifestyle. MAGI Balkans recently focused on diagnosis and research of rare genetic diseases, offering counselling and genetic testing to Albanian patients.

The laboratory uses next-generation sequencing (NGS) technology, which has become cheaper and faster in the last 10 years, to diagnose various genetic disorders. NGS has replaced Sanger sequencing as the elective method, especially for diseases with very heterogeneous phenotypes, alleles and loci. Protocol standardization has facilitated availability of these tests in low to medium income countries. Here we report tests performed in collaboration with MAGI Euregio, Italy, for the diagnosis of rare genetic diseases in Albanian patients and their families.

Methods

Patients were enrolled in a genetic screening program from April 2016 to September 2017. Genetic analysis on DNA extracted from whole blood was carried out after obtaining informed consent from patients.

A next generation sequencing (NGS) approach was used to analyze all known disease-associated genes in parallel, substantially increasing the detection rate while optimising time- and cost-effectiveness. Targeted resequencing was performed using the Illumina commercial kit, TruSight One Sequencing Panel, on an Illumina MiSeq platform. This kit was designed for genomic analysis of the coding regions of 4813 genes with associated clinical phenotypes (http://www.illumina.com/products/trusight-one-sequencing-panel.ilmn). In-solution target enrichment was performed according to the manufacturer's protocol. Briefly, 50 ng of genomic DNA was simultaneously fragmented and tagged by Nextera transposon-based shearing technology. A 3-plex sample library pool was sequenced using a 150 bp paired-end reads protocol on a MiSeq sequencer (Illumina, San Diego, CA).

The genes analyzed as associated with patient phenotypes were defined by literature search and by consulting databases such as OMIM (https://omim.org) and Orphanet.

Bioinformatic analysis of the data was performed using an in-house pipeline to align sequence reads with a reference genome, with variant calling, annotation and variant filtering to remove benign single nucleotide polymorphisms (variants with allele frequency ≤ 0.03). Public databases such as 1000 Genomes Project Database (http://www.internationalgenome.org/), Exome Variant Server (EVS) database (http://evs.gs.washington.edu/EVS/), Exome Aggregation Consortium (ExAC) database (www.exac.broadinstitute.org) and the public database of single nucleotide variants (dbSNP, www.ncbi.nlm.nih.gov/SNP/) were used to filter and prioritize the variants and to check for allele frequencies, while the Human Gene Mutation Professional Database (HGMD) (http://www.biobase-international.com/product/hgmd) was used to identify genetic variations previously reported as pathogenic. In silico prediction software, such as SIFT (Sorting Intolerant From Tolerant), PolyPhen2 (Polymorphism Phenotyping v2), Mutation Taster, MutationAssessor and LRT (Likelihood Ratio Test) were used to assess potential deleterious effects of missense variants. Wild-type amino acid properties were compared with the variations (http://www.russelllab.org/aas/aas.html). The criteria applied to evaluate the pathogenic nature of selected variants were according to Richards et al. (5). Literature and databases were checked for previous descriptions of selected candidate variants.

Genetic variation was confirmed by PCR coupled with direct Sanger sequencing of target regions on a CEQ8800 Sequencer (Beckman Coulter) according to the manufacturer's protocols.

Results

A total of 7 probands and 10 additional family members from different cities across Albania (**Fig. 1**) were screened by NGS.

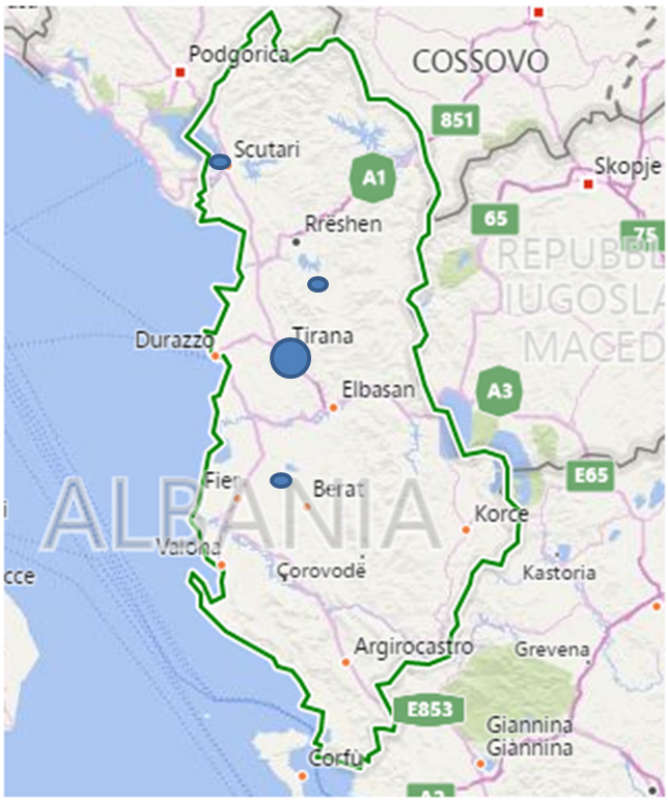

Figure 1. Blue dots represents proband and family members from different cities.

The probands manifested ichthyosis, familial adenomatous polyposis (FAP), diabetes, syndromic craniosynostosis, fronto-temporal dementia, fragile X syndrome or ataxia. For 3/7 probands we obtained inconclusive results, whereas for the other four we identified pathogenic or likely pathogenic variants. The variants were confirmed by Sanger sequencing. Segregation analysis twas performed on the DNA of available family members. We obtained an overall detection rate of 57%. The variants identified were entire *STS* gene deletion in the patient with ichthyosis, a stop variant in APC in the FAP patient, and missense variants in *UBQLN2* in the FTD patient and in *TWIST1* in the craniosynostosis patient.

Conclusion

Molecular diagnosis is of great importance for clinical management of patients, and genetic testing by NGS is the elective choice for a wide variety of disorders with complex or overlap-

ping phenotypes (6). The advent of targeted NGS sequencing led to the development of assays readily adapted to the screening of different phenotypes in an affordable and fast way. The development of such services in low-middle income countries is raising awareness and increasing the availability of genetic testing outside major cities, with great advantages for patients and their families in terms of access to available healthcare resources (i.e. clinical trials and therapies), recurrence-risk counseling, and informed reproductive choices for affected family members (2).For example, screening in one family led to identification of a family member carrying the same pathogenic variant as the proband. This patient was included in a regular surveillance program to monitor for onset of the disease in order to avoid more serious consequences. For probands whose molecular analysis failed to identify the cause of the disease, we suggested copy-number variation evaluation by SNP array or whole exome sequencing of at least patient and parents to assess the possibility of variants in genes not examined by our approach.

Conflicts of interest

The authors declare that they have no competing interests or conflicts of interest in relation to this paper.

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