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Research paper

Usher syndrome: An effective sequencing approach to establish a genetic and clinical diagnosis

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ABSTRACT

Usher syndrome is an autosomal recessive disorder characterized by retinitis pigmentosa, sensorineural hearing loss and, in some cases, vestibular dysfunction. The disorder is clinically and genetically heterogeneous and, to date, mutations in 11 genes have been described. This finding makes difficult to get a precise molecular diagnosis and offer patients accurate genetic counselling. To overcome this problem and to increase our knowledge of the molecular basis of Usher syndrome, we designed a targeted resequencing custom panel. In a first validation step a series of 16 Italian patients with known molecular diagnosis were analysed and 31 out of 32 alleles were detected (97% of accuracy). After this step, 31 patients without a molecular diagnosis were enrolled in the study. Three out of them with an uncertain Usher diagnosis were excluded. One causative allele was detected in 24 out 28 patients (86%) while the presence of both causative alleles characterized 19 patients out 28 (68%).

Sixteen novel and 27 known alleles were found in the following genes: *USH2A* (50%), *MYO7A* (7%), *CDH23* (11%), *PCDH15* (7%) and *USH1G* (2%). Overall, on the 44 patients the protocol was able to characterize 74 alleles out of 88 (84%).

These results suggest that our panel is an effective approach for the genetic diagnosis of Usher syndrome leading to: 1) an accurate molecular diagnosis, 2) better genetic counselling, 3) more precise molecular epidemiology data fundamental for future interventional plans.

1. Introduction

Usher syndrome (USH) is an autosomal recessive disorder characterized by the association of visual loss due to retinitis pigmentosa (RP), sensorineural hearing loss (SNHL) and in some cases, vestibular dysfunction. The disease has a prevalence of approximately 3.2–6.2/100,000 or even more (Kimberling et al., 2010), accounting for more than 50% of deaf-blind cases, about 18% of RP cases, and 5% of all cases of congenital deafness (Yan et al., 2010).

The disorder is clinically and genetically heterogeneous and USH was historically divided into three subtypes, according to disease severity and progression. Type I (USH1) is the most severe form characterized by profound congenital hearing loss, prepuberal onset of RP, and vestibular dysfunction. Type II (USH2) is characterized by moderate to profound (sloping pattern) congenital hearing loss, later onset of RP and normal vestibular function (Besnard et al., 2014). Finally, type III (USH3), the less common form with a prevalence of 2–4% among Usher patients (Yan et al., 2010) displays variable a) onset of progressive hearing loss, b) onset of RP and c) vestibular function (normal to absent). This classification of USH remains in clinical use, but atypical cases have been described

Abbreviations: USH, Usher syndrome; RP, retinitis pigmentosa; SNHL, sensorineural hearing loss; USH1, Usher syndrome Type I; USH2, Usher syndrome Type II; USH3, Usher syndrome Type III; TRS, targeted resequencing; ASHA, American Speech-Language-Hearing Association; PGM, Personal Genome Machine; VCF, Variant Call Format; IGV, Integrative Genomics Viewer; HSF, Human Splicing Finder; MAF, minor allele frequency

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(Yan et al., 2010). To date, at least 12 genetic loci have been mapped for the three types of USH and 11 genes have been identified so far (Bonnet et al., 2012). In particular, seven loci have been described for type 1 (*USH1B–USH1J*), three for type 2 (*USH2A*, *USH2C* and *USH2D*) and only one for type 3.

Due to the high degree of genetic and clinical heterogeneity, genetic screening with traditional methods such as direct sequencing or genotyping microarrays (i.e. able to detect only a given number of mutations) is challenging. Recently, targeted or whole exome sequencing was shown to be a powerful tool for discovering novel disease-related genes or genetic mutations in large genomic regions (Bowne et al., 2011). In this light, to increase our knowledge on the molecular basis of USH and to provide an accurate and reliable molecular diagnosis for USH patients, we developed a targeted resequencing (TRS) protocol for the simultaneous analysis of USH genes, demonstrating its usefulness in discovering novel alleles as well as in routine molecular diagnosis which in turn opens up for new perspectives in the clinical assessment of these patients.

2. Methods

2.1. Patients

In our study we recruited 47 individuals affected by a combination of hearing loss and visual impairment from different Italian clinical genetics centres. Specifically, 16 patients that had enrolled at the U.O. Genetica Medica Policlinico S. Orsola – Malpighi of Bologna and that had previously been characterized clinically and genetically as Usher patients, were used to validate our USH custom panel. The remaining 31 patients came from Bologna (see above), IRCSS Burlo Garofolo (Trieste) and Fondazione IRCSS Ca' Granda Ospedale Maggiore (Milano). Moreover, in order to confirm mutation segregation, first degree relatives were genotyped wherever possible. Hearing loss was evaluated in line with the American Speech-Language-Hearing Association (ASHA) (Clark, 1981). All participants underwent a vestibular test, with evaluation of spontaneous and evoked eye movements as well as a caloric test. Thorough ophthalmic exams were performed, including measurement of central visual acuity, a fundus exam and electroretinography. Information on ethnic background was not provided, but all subjects were of Italian origin. Blood samples were drawn for DNA extraction. All patients provided written informed consent for both genetic counselling and molecular genetic testing prior to enrolment. In the case of minors/children, a written informed consent was obtained from the next of kin. A total of 13 females (42% – mean age 51 years old) and 18 males (58% – mean age 37 years old) were enrolled in our study. The mean age of USH1 was less than 15 years old, whereas, in USH2, it was less than 28 years old (Table 1).

The study was performed in accordance with the tenets of the Declaration of Helsinki and the ethical guidelines of our institution.

2.2. DNA extraction

Genomic DNAs were extracted from peripheral whole blood using the QIAsymphony DSP DNA midi kit v1 and QIAsymphony robotic device (Qiagen, Milan, Italy) following manufacturer's instructions. DNA sample were stored at −20 °C until use. DNA integrity was evaluated with 1% agarose gel electrophoresis. DNA concentration was measured with a Nanodrop ND1000 spectrophotometer (Thermo Fisher Scientific, DE, USA). These concentrations were confirmed with Qubit fluorometer (Life Technologies, CA, USA).

Table 1
Clinical features of Usher patients. USHER ID: ID of each patient; Degree: severity of hearing loss; Visual field: type of visual impairment, RP: Retinitis Pigmentosa; Balance: vestibular function. N/A: Not available; *: Uncertain Usher diagnosis.

Hearing loss					Visual impairment		
Usher ID	Sex	Subtype	Degree	Onset	Visual field	Onset	Balance
USH 17	F	Usher 1	Profound	Congenital	RP	2nd decade	Areflexia
USH 18	M	Usher 2	Moderate-Severe	Infant	RP	2nd decade	Normal
USH 19	M	Usher 1	Profound	Congenital	RP	4th decade	Areflexia
USH 20	M	Usher 1	Profound	Infant	RP	3rd decade	Areflexia
USH 21	F	Usher 1	Profound	N/A	RP	N/A	Areflexia
USH 22	M	Usher 2	Moderate	2nd decade	RP	2nd decade	Normal
USH 23	M	Usher 1	Profound	Congenital	RP	2nd decade	Areflexia
USH 24	M	Usher 2	Moderate-Severe	N/A	RP	N/A	Normal
USH 25	F	Usher 2	Moderate-Severe	N/A	RP	N/A	Normal
USH 29	M	Usher 1	Profound	Congenital	RP	Infant	Areflexia
USH 32	F	Usher 1	Profound	Congenital	RP	Infant	Areflexia
USH 36*	F	Usher 2	Mild	6th decade	RP	4th decade	Normal
USH 45	F	Usher 1	Profound	Congenital	RP	Infant	Areflexia
USH 46	M	Usher 2	Moderate	Infant	RP	2nd decade	Normal
USH 48*	F	Usher 2	Moderate	6th decade	RP	3rd decade	Normal
USH 49	M	Usher 2	Moderate	3rd decade	RP	4th decade	Normal
USH 52	F	Usher 2	Moderate-Severe	2nd decade	RP	2nd decade	Normal
USH 53	M	Usher 1	Profound	N/A	RP	2nd decade	Areflexia
USH 54	M	Usher 2	Severe	2nd decade	RP	2nd decade	Normal
USH 60	F	Usher 2	Moderate-Severe	Congenital	RP	2nd decade	Normal
USH 62*	M	Usher 2	Moderate-Severe	4th decade	RP	3rd decade	Normal
USH 63	M	Usher 2	Moderate-Severe	2nd decade	RP	3rd decade	Normal
USH 66	F	Usher 1	Severe	Congenital	RP	2nd decade	Areflexia
USH 67	F	Usher 2	Moderate-Severe	Infant	RP	6th decade	Normal
USH 68	M	Usher 2	Moderate	N/A	RP	4th decade	Normal
USH 69	F	Usher 1	Profound	Congenital	RP	N/A	Areflexia
USH 83	F	Usher 2	Moderate	2nd decade	RP	2nd decade	Normal
USH 84	M	Usher 2	Moderate	Congenital	RP	3rd decade	Normal
USH 85	M	Usher 2	Moderate	Congenital	RP	4th decade	Normal
USH 86	M	Usher 2	Moderate	Infant	RP	2nd decade	Normal
USH 87	M	Usher 2	Moderate	Infant	RP	2nd decade	Normal