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Expanding the clinical and mutational spectrum of germline *ABL1* mutations-associated syndrome
A case report

Nereida Bravo-Gil, PhD[a,b], Irene Marcos, PhD[a,b], Antonio González-Meneses, MD[c], Guillermo Antiñolo, MD, PhD[a,b,*], Salud Borrego, MD, PhD[a,b,*]

Abstract
Rationale: Clinical and genetic management of patients with rare syndromes is often a difficult, confusing, and slow task.

Patient concerns: Male child patient with a multisystemic disease showing congenital heart defects, facial dysmorphism, skeletal malformations, and eye anomalies.

Diagnosis: The patient remained clinically undiagnosed until the genetic results were conclusive and allowed to associate its clinical features with the germline *ABL1* mutations-associated syndrome.

Interventions: We performed whole-exome sequencing to uncover the underlying genetic defect in this patient. Subsequently, family segregation of identified mutations was performed by Sanger sequencing in all available family members.

Outcomes: The only detected variant compatible with the disease was a novel heterozygous nonframeshift de novo deletion in *ABL1* (c.434_436del; p.Ser145del). The affected residue lays in a functional domain of the protein, it is highly conserved among distinct species, and its loss is predicted as pathogenic by in silico studies.

Lessons: Our results reinforce the involvement of *ABL1* in clinically undiagnosed cases with developmental defects and expand the clinical and genetic spectrum of the recently reported *ABL1*-associated syndrome. In this sense, we described the third germline *ABL1* causative mutation and linked, for the first time, ocular anterior chamber anomalies to this pathology. Thus, we suggest that this disorder may be more heterogeneous than is currently believed and may be overlapping with other multisystemic diseases, hence genetic and clinical reassessment of this type of cases should be considered to ensure proper diagnosis.

Abbreviations: BWA = Burrows-Wheeler Alignment, GATK = Genome Analysis Toolkit, HSF = Human Splicing Finder, OFC = occipitofrontal circumference, PPS = Peters-plus syndrome, RDs = rare diseases, WES = whole-exome sequencing.

Keywords: ABL1, developmental defects, next-generation sequencing, rare diseases, whole-exome sequencing

1. Introduction

Rare diseases (RDs) are defined by an incidence of <1 case per 2000 individuals. Currently, >7000 RDs have been reported, but only 100 of these conditions account for 80% of cases.[1] The low frequency, together with the partial overlapping of clinical features, means that diagnosis of these cases is often a difficult, confusing, and slow task, which, in many cases, leads to misdiagnosis. In this regard, considering that about 80% of RDs have a genetic basis,[2] genetic testing is not only crucial, but also challenging, to achieve a decisive and precise diagnosis. The next-generation sequencing technologies are very helpful in the molecular diagnosis of RDs.

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Specifically, whole-exome sequencing (WES) allows the identification of new disease-associated genes, leading to new phenotypic associations and rectifying discrepancies between the underlying genetic defect and clinical manifestations.[3]

This situation becomes even more pronounced in rare multisystemic syndromes, in which clinical findings can point in many different directions, the phenotypic variability is even higher than in other RDs and not all organs begin to be affected simultaneously. Recently, an article has been published identifying germline mutations in the *ABL1* gene (MIM: 189980) in 6 cases belonging to 4 families with a previously unreported autosomal dominant syndrome characterized by congenital heart defects, dysmorphic facial features, and skeletal malformations (MIM: 617602), among others.[4] Thus, that article associates, for the first and only time, germline *ABL1* mutations with a genetic disorder, suggesting that this gene has a key role during development. *ABL1* is a protooncogene that belongs to the Abelson family of cytoplasmic nonreceptor tyrosine kinases, which have been implicated in the regulation of cytoskeletal processes.[5,6] In this light, given the extensive functions of protooncogenes, it is not surprising that germline changes in these genes are not only related to cancer, but they may also be involved in other human diseases.[7,8]

Herein, we applied WES to uncover the genetic cause of a male child patient with a multisystemic syndrome in which age and clinical features made it extremely difficult to make an accurate clinical diagnosis. This approach allowed us to identify a novel de novo mutation in the *ABL1* gene (c.434_436del; p.Ser145del) as the most likely genetic cause of the disease in this patient.

2. Methods

2.1. Subjects and clinical assessment

The patient was derived from the Department of Pediatrics to our Department. In addition, available family members (mother, father, and brother) were included into the study. All subjects underwent peripheral blood extraction for genomic DNA isolation from leukocytes using Chemagic 360 (Chemagen). The study was performed in accordance with the tenets of the Declaration of Helsinki, and all experimental protocols were approved by the Institutional Review Boards of the University Hospital Virgen del Rocio (Seville, Spain). Before analysis, written informed consent was obtained from all participants or their respective legal representative. Clinical description was obtained by reviewing medical records.

2.2. Previous molecular genetic assays

DNA sample from the index patient was first analyzed and excluded for large rearrangements by applying karyotype and CGH array.

2.3. Whole-exome sequencing and data analysis

Library preparation and exome capture were performed according to the manufacturer's protocol (NimbleGen SeqCap EZ Library SR v5.1) for sequencing on Illumina platforms and using the SeqCap EZ MedExome kit (NimbleGen). Sequencing was carried out by a NextSeq 500 instrument (Illumina) and a NextSeq High-output v2 (300 cycles) reagent kit.

Reads from WES were aligned by Burrows-Wheeler Alignment (BWA). Quality control parameters were checked using the

BEDtools package. After duplicates removal, variant calling and filtering were performed with the software Genome Analysis Toolkit (GATK), and reads with coverage <20X and strand bias (FS>60.0) were discarded. The remaining variants were annotated with ANNOVAR. Annotated variants were semiautomatically prioritized based on their MAF in public databases (dbSNP, 1000 Genomes, EVS, GnomAD and CIBERER Spanish Variant Server),[9] considering <0.01 as a suitable frequency for variants matching an autosomal recessive inheritance mode and <0.0001 for those that fit with an autosomal dominant inheritance pattern; their heterozygous/homozygous states; their effects on the protein sequence; known clinical associations of the mutated gene; and ClinVar and HGMD entries. Finally, the coverage command of BEDtools and subsequent normalization were used for the detection of Copy Number Variations.

2.4. Pathogenicity assessment and variant validation

Candidate variants were confirmed by Sanger sequencing and cosegregation analyses were performed in available family members. We used Polyphen-2, SIFT, Mutation Taster, and Human Splicing Finder (HSF) scores to predict the potential impact of variants on the function of the encoded protein. Mutalyzer was used to adjust the nomenclature of variants to the Human Genome Variation Society guidelines. Finally, the degree of conservation of the amino acids was determined by using the multiple sequence alignment option of Clustal Omega.

3. Case report

3.1. Clinical characteristics

The index patient was born at 38 weeks, being the second child of healthy parents and the only affected individual of the family. Intrauterine growth restriction was observed during pregnancy. At birth, he presented a weight of 2020 g and a length of 46 cm, with an occipitofrontal circumference (OFC) of 32.5 cm and Apgar scores of 6/9/10. At 8 months, the patient's weight was 6 kg (<P1, −2.52DE) with a length of 64 cm (<P1, −2.79DE), and an OFC of 40.2 cm (<P1, −4.52DE). The patient showed manifestations according to a multisystemic syndrome characterized by different clinical features, as shown in Table 1. He had been born with double outlet right ventricle with normal position

Table 1

Clinical findings in the index patient compared with previous *ABL1*-associated features.

	Our index patient	*ABL1* syndrome[4]
Mode of inheritance	Autosomal dominant	Autosomal dominant
Facial dysmorphism	+	+
Congenital heart defects	+	+
Skeletal malformations	+	+
Joint problems	-	+ (83%)
Developmental delay	+	+ (83%)
Intrauterine growth restriction	+	+
Gastrointestinal problems	+	+ (83%)
Male genital/sexual abnormalities	+	+ (75%)
Ocular anterior chamber anomalies	+	−

Clinical characteristics previously associated with *ABL1* mutations correspond to those reported by Wang et al. The symbol "+" shows the presence of each clinical feature in the corresponding patient or group of patients, whereas the symbol "−" means their absence. Points with "+" are in the 100% of cases or, alternatively, the value indicated in parentheses.
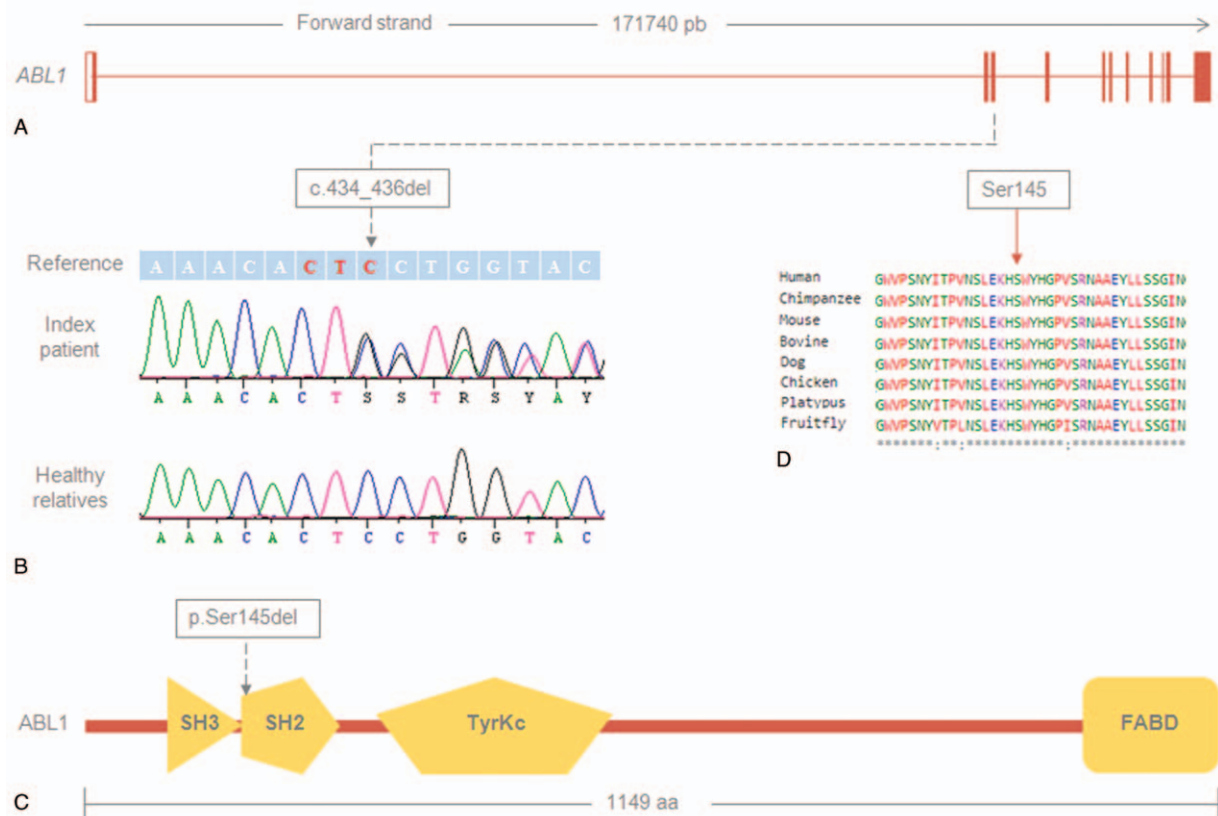
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Figure 1. Schematic representation of the novel *ABL1* mutation. (A) *ABL1* gene structure is composed of 11 exons (vertical lines). The mutation identified in the exon 3 of this gene is indicated with a broken line. (B) Electropherograms illustrating the heterozygous *ABL1* variant and the wild type sequence observed in healthy relatives. (C) Protein depiction of the consensus secondary structure of ABL1 (NM_007313) according to the SMART (Simple Modular Architecture Research) tool, showing the SH3, SH2, tyrosine kinase catalytic (TyrKc), and F-actin binding (FABD) domains. The location of the affected residue (Ser145) is stated with a broken line. (D) Alignment of ABL1 orthologs protein sequences. The mutated residue is marked by an arrow.

of the vessels, pulmonary valve stenosis and hypoplasia of the pulmonary trunk and branches. He also showed dysmorphic facial features, including thin upper lip, smooth philtrum, small nose, micrognathia, synophridia, and microcephaly. Ophthalmological evaluation revealed bilateral ocular anterior chamber anomalies and sclerocornea. Furthermore, he had umbilical hernia, micropenis with hypoplastic scrotum, mild developmental delay, and hypotonia.

3.2. Identification of candidate variants

WES resulted in a mean coverage of 120.4× and a percentage of reads on target of 74.1%. Filtering, bioinformatic analysis, and semiautomatic prioritization highlighted a heterozygous novel nonframeshift deletion (c.434_436del; p.Ser145del) in the exon 3 of *ABL1* (NM_007313) (Fig. 1A). Family segregation showed that it was a de novo change because it was not inherited from any of its parents, being the affected member the only one that carried it (Fig. 1B). Thus, this variant is compatible with the disease phenotype as an autosomal dominant de novo mutation. Remarkably, no other potentially causal variants in other genes were found. The candidate-identified variant was submitted to the public gene variant database, Leiden Open Variation Database 3.0 (http://www.lovd.nl/3.0/home, Variant ID #0000368992).

3.3. Pathogenicity evaluation of the ABL1 mutation

The amino acid Serine at position 145 of the protein lays in the SH2 domain of ABL1 (Fig. 1C), being the second residue that constitutes this conserved region. In addition, Ser145 is highly conserved among distinct species, from human to fruit fly (Fig. 1D). Applicable in silico functional studies for deletion-type mutations predicted that the *ABL1* variant c.434_436del was likely deleterious. On the one hand, Mutation Taster classified this mutation as disease-causing due to the modification of the amino acid sequence, the possible loss of the SH2 domain leading to the alteration of protein features and the potential change on the splicing process. On the other hand, the specific splicing prediction tool HSF also indicated that this *ABL1* mutation could alter the canonical splicing of the mRNA, generating a new donor splice site with a score of 72.1 and a variation of 116.84%.

4. Discussion

The high phenotypic variability of patients with RDs, especially for multisystemic syndromes, greatly complicates their clinical and genetic diagnosis. Here, the only detected variant compatible with the disease was a de novo mutation in *ABL1* (c.434_436del; p.Ser145del), reinforcing the role of germline *ABL1* mutations as a nonnegligible cause of multisystemic syndromes characterized by dysmorphic features, heart defects, and skeletal abnormalities.

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Moreover, we suggest that the involvement of this gene in clinically undiagnosed cases with developmental defects, although rare, could be underestimated because *ABL1* is not a routinely screened gene for these conditions. This also highlights the importance of hypothesis-free approaches such as WES to address this type of cases and the relevance of having an accurate genetic diagnosis for the appropriate management of patients.

The clinical picture showed by the index patient was consistent with the recently reported unidentified syndrome caused by *ABL1* variants[4] (Table 1), sharing the vast majority of clinical features except with regard to ocular development. These results expand the clinical and genetic spectrum of this disorder because, to date, only 2 germline *ABL1* mutations have been identified as causative and it is the first time to be linked to ocular anterior chamber anomalies. The phenotypic variability among affected patients (Table 1) may be related to the detected mutation and its effect on the protein function. Otherwise, the phenotypic overlap of multisystemic diseases and the diffuse limits among clinical entities mean that we cannot rule out that we are facing a new manifestation of a previously known syndrome with a challenging differential diagnosis. In fact, clinical features of the index patient could be also overlap with Peters-plus syndrome (PPS),[10] an autosomal recessive RD caused by *B3GLCT* mutations and characterized by dysmorphic features, skeletal malformations, developmental delay, and eye abnormalities, among others.[11] However, mutations in this gene were not found, discarding this possibility.

The identified nonframeshift *ABL1* deletion (c.434_436del) causes the loss of a serine at position 145 (Ser145) of the protein. This residue lays in the SH2 domain of the protein, a region responsible for the specific binding to tyrosine-phosphorylated peptides.[12] The crystal structure of ABL1 revealed that a phosphoserine residue at position 69 forms hydrogen bonds with Ser145 in the SH3-SH2 connector, whose alteration leads to an increase in kinase activity.[13] Thus, mutation p.Ser145del could be increasing the phosphorylation of specific ABL1 substrates, which is consistent with the reported effect for the other disease-associated ABL1 variants.[4] Therefore, given the known relevance of the conserved Ser145 in protein function and structure, family cosegregation and genotype–phenotype concordances, the novel ABL1 mutation p.Ser145del was considered the most likely genetic cause of the disease in this patient. In addition, there seems to be a correspondence between the disease phenotype and the type of causative mutation because none disease-associated *ABL1* mutations results in a prematurely truncated protein. This association between autosomal dominant disorders and a priori less damaging mutations, such as missense and nonframeshift variants, has already been described for other genes and diseases, in which it has been suggested that these changes cause a gain of function of the protein with further deleterious effects.[14]

In summary, this report reinforces the role of *ABL1* in congenital development defects and expands the clinical and genetic spectrum of the recently reported unidentified syndrome associated with germline *ABL1* mutations, contributing a third pathogenic variant and adding eye anomalies as a further disease-associated feature. Furthermore, we suggest that genetic and clinical reassessment of other cases with overlapping multi-systemic diseases should be considered to ensure proper diagnosis, bearing in mind that this condition may be more heterogeneous than is currently believed and may be under-estimated with all implications this entails.

Author contributions

Conceptualization: Guillermo Antiñolo, Salud Borrego.
Data curation: Antonio González-Meneses.
Formal analysis: Nereida Bravo-Gil.
Funding acquisition: Guillermo Antiñolo, Salud Borrego.
Investigation: Nereida Bravo-Gil, Irene Marcos.
Methodology: Nereida Bravo-Gil, Irene Marcos.
Resources: Guillermo Antiñolo, Salud Borrego.
Supervision: Guillermo Antiñolo, Salud Borrego.
Validation: Irene Marcos.
Writing – original draft: Nereida Bravo-Gil, Irene Marcos.
Writing – review and editing: Antonio González-Meneses, Guillermo Antiñolo, Salud Borrego.
Salud Borrego orcid: 0000-0002-2513-6161.

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