European Journal of Medical Genetics 59 (2016) 330–336

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

A novel *ACVR1* mutation detected by whole exome sequencing in a family with an unusual skeletal dysplasia

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ARTICLE INFO

Article history:
Received 10 January 2016
Received in revised form
18 April 2016
Accepted 11 May 2016
Available online 13 May 2016

Keywords:
Whole exome sequencing
ACVR1
Skeletal dysplasia
Fibrodysplasia ossificans progressiva
FOP variant

ABSTRACT

"Disorganized Development of Skeletal Component" (DDSC) is a group of genetic skeletal dysplasia, caused by mutations in 9 genes including *ACVR1*. The most known *ACVR1*-related disorder is fibrodysplasia ossificans progressiva (FOP). FOP variants are frequently encountered with diagnostic challenges due to overlapping clinical manifestations and variable severity. Application of high throughput sequencing methods can overcome these limitations by simultaneous investigation of the entire *ACVR1* gene together with other genes involved in disorders with similar manifestations. A 33-year-old man with an unusual skeletal dysplasia and no previous clinical diagnosis is presented in this study. Whole exome sequencing detected a novel c.737T>A (p.Phe246Tyr) mutation in *ACVR1* gene. Detailed targeted variant analysis in 226 known genes associated with genetic skeletal disorders together with more specific targeted analysis in 9 genes associated with DDSC ruled out the involvement of other investigated genes. Proband's phenotypically normal father and brother had the same mutation in whom subsequent investigations showed subclinical radiographic findings.

The clinical manifestations, the disease course, and the molecular findings of involvement of *ACVR1* gene in this family are suggestive of "FOP variant" or an unusual *ACVR1*-related skeletal dysplasia. Moreover, this report has demonstrated the critical role of the next generation sequencing technique in characterizing such a rare disorder with variable and even no clinical manifestations, providing the opportunity for effective preventive measures such as preimplantation genetic diagnosis.

1. Introduction

"Disorganized Development of Skeletal Component" (DDSC) is a group of genetic skeletal dysplasia causing abnormal development of skeletal components such as exostoses, ecnhondromas, and ectopic calcification (Warman et al., 2011). Nine genes including *ACVR1* have been reported to be associated with DDSC. The most known *ACVR1*-related disorder is Fibrodysplasia ossificans progressiva (FOP) which involves one of every two million people (Gregson et al., 2011; Miao et al., 2012). It is inherited in an autosomal dominant mode of inheritance. Most cases arise sporadically with only a few reported familial cases (Morales-Piga et al., 2012; Shore et al., 2006). Patients with "classic" features of FOP, develop heterotopic ossification of tendons, ligaments, skeletal muscles and/or fascia, usually in childhood with no smooth or cardiac muscle involvement. Heterotopic ossification is usually triggered by minor trauma and viral illness and occurs following a period of soft tissue swelling and inflammation (Gregson et al., 2011). Congenital great toe malformations are the earliest most recognizable feature of 'classic' FOP. However, in 2009, Kaplan et al. reported on patients with clinical features unusual for FOP. These atypical FOP patients included two classes: "FOP-plus" (classic defining features of FOP

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http://dx.doi.org/10.1016/j.ejmg.2016.05.007
1769-7212/

plus one or more additional features that are not commonly associated with FOP) and "FOP variant" in which there are major variations in the classic defining features of FOP (Kaplan et al., 2009). FOP is commonly misdiagnosed, leading to unnecessary diagnostic biopsies that exacerbate progression of the condition (Kaplan et al., 2008a; Kitterman et al., 2005). Atypical forms of FOP, especially the FOP variant, are much more frequently encountered with such unnecessary and even harmful diagnostic and therapeutic procedures due to overlapping clinical manifestations and variable severity. (Kaplan et al., 2008b; Zhang et al., 2013). Application of high throughput DNA sequencing methods can overcome these limitations by simultaneous investigation of the entire *ACVR1* gene together with other genes involved in disorders with similar manifestations (Liu et al., 2015).

Here, we report on a family with an unusual skeletal dysplasia in which, no definite diagnosis had been previously made based on standard clinical and para-clinical investigations. Whole exome sequencing followed by a comprehensive stepwise variant analysis has been applied to investigate the underlying genetic cause of such unusual skeletal dysplasia.

2. Case presentation

The proband (Fig. 1, individual 2:2), a 33-year-old man with dysplasia of multiple bones and azoospermia (with successful sperm recovery through testicular sperm extraction), was referred for genetic counseling to discuss about the recurrence risk and possible preventive measures.

His past medical history revealed multiple episodes of spontaneous swellings at multiple sites, including hands and skull. He was firstly noted at 5 years of age to have enlargement of the second and third fingers of the right hand. Thereafter, deformity of the forth digit and multiple soft tissue tumor-like swellings in the left hand occurred. Progression of the disease was slow and evanescent until 15 years of age when other fingers were also involved; subsequent ankylosis of the joints occurred and overgrowth of the skeletal bones -possibly due to the heterotopic ossification-continued in the skull (frontal and occipital bones). He had clinically normal great toes at birth and during the disease course. He had no bone or soft tissue involvement in the other parts of the body. Surgical correction of the deformity of the forth finger and resection of large soft tissue swellings in the left hand were respectively attempted at 8 and 18 years of age. No substantial progression of bony overgrowths occurred afterward.

At 19 years of age he was noted as having multiple benign tumoral lesions in both testicles which were surgically resected during two separate operations. According to the patient, the histopathologic diagnosis was reported as "tumor of the epididymis". However, no medical document was provided for verification. There was also a history of a hyperpigmented patch found on the left arm (extending from shoulder to elbow) that, together with soft tissue swellings, had been reportedly (by the patient) proposed as "neurofibromatosis" in a differential diagnosis at the early stage of the disease.

The patient had therefore been diagnosed as having multiple enchondromatosis (Ollier disease) or Maffucci syndrome because of the mentioned involvement of multiple bones.

At physical examination the proband had severe digital dysplastic lesions in both hands together with two bony overgrowths in the skull (frontal and occipital bones) (Fig. 2). Details of radiographic findings in both hands are shown in Fig. 2C. No X-ray corresponding to the earlier stages of the disease (proband's childhood) was available.

The patient also reported on an uncle (individual 1:1, aged 66 years old) with several bony overgrowths in the skull and no further bone involvement, whom declined to participate in further clinical examination and molecular genetic investigations. Other family members were clinically unaffected.

3. Materials and methods

Through several comprehensive genetic counseling sessions, the benefits and limitations of all possible genetic investigations including targeted genetic testing for proposed diagnoses as well as using high throughput sequencing techniques were thoroughly discussed. As no definite diagnosis had been made, the patient opted to do whole exome sequencing to reveal the underlying genetic cause. A written approved informed consent was then obtained.

Whole exome sequencing was carried out on DNA sample of the

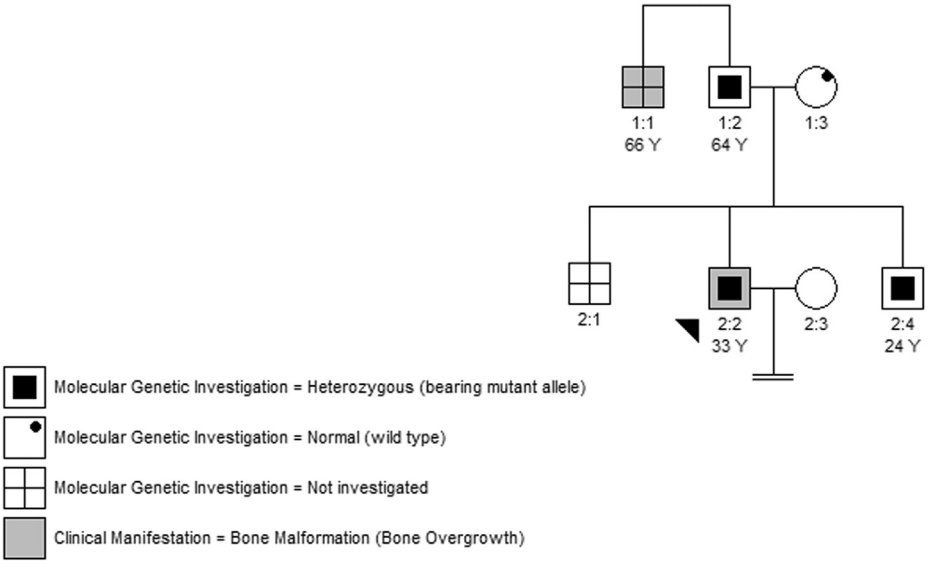

Fig. 1. Family pedigree. Arrow head denotes the propositus.