Epidemiology and Phenotypic Characteristics of Dual Molecular Diagnosis Cases in Skeletal Abnormality

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Abstract

Background

Skeletal abnormality is a heterogeneous group of disorders that affects the composition and structure of bone and cartilage. In our previous studies, we have revealed that a substantial proportion of cases with early-onset scoliosis could be explained by monogenic disorders such as Marfan syndrome and Ehlers-Danlos syndrome. More recently complex phenotypes caused by more than one genetic defect (i.e., dual molecular diagnosis) have also been reported in skeletal abnormalities. To explore the molecular epidemiology and phenotypic characteristics of dual diagnosis in skeletal abnormalities, we described cases with dual molecular diagnosis from the Deciphering disorders Involving Scoliosis and COmorbidities (DISCO) study.

Results

In total, 1108 patients with skeletal abnormality from the DISCO study underwent Exome Sequencing. We identified eight probands with dual molecular diagnosis, including three (0.7%) from individuals with early-onset scoliosis (EOS), three (0.5%) from individuals with short stature, and two (2%) from individuals with congenital hand/foot deformity (CHFA). Other skeletal abnormalities observed in these individuals included bone fracture and interphalangeal joint contracture. All the eight probands have dual diagnosis of two autosomal dominant (AD) diseases. A total of 16 variants in 12 genes were identified. A substantial rate (5 of 10) of the identified causal variants were of *de novo* origin. The frequently observed molecular diagnoses (observed in more than one patient) include Osteogenesis Imperfecta Type I (*COL 1A1*, MIM:166200), Neurofibromatosis, Type I (*NF1*, MIM:162200) and Marfan Syndrome (*FBN1*, MIM:154700). These patients with dual molecular diagnosis often present blended phenotypes of two diseases, which significantly complicate their diagnostic process.

Conclusions

This study revealed the molecular epidemiology and complex diagnostic odyssey of dual molecular diagnosis through analyzing the clinical traits of skeletal abnormalities in eight patients.

Background

Skeletal abnormality is a heterogeneous group of disorders that affects the composition and structure of bone and cartilage (1). Phenotypes involved in skeletal abnormality include short stature, scoliosis, joint dislocation, limb deformities, and bone density changes etc. According to the tenth version of the Nosology and Classification of Genetic Skeletal Disorders, there are 461 different diseases that are classified into 42 groups based on their clinical, radiographic, and/or molecular findings (2). Genetic factors play an important role during the pathogenesis of skeletal abnormalities. In our previous studies, we have revealed that a substantial proportion of cases with early-onset scoliosis could be explained by monogenic disorders such as Marfan syndrome (MIM:154700) and Ehlers-Danlos syndrome (MIM:130000) (3).

In addition to monogenic conditions, complex phenotypes caused by more than one genetic defect (i.e. dual molecular diagnosis) have also been reported in skeletal abnormalities. For example, Aggarwal et al. reported a fetus with complex joint dislocations and congenital scoliosis who was detected to be double heterozygote for putatively pathogenic Fibrillin-1 (*FBN1*) and Fibrillin-2 (*FBN2*) variants (4). Tang et al. identified a patient with pathogenic variants in both *FBN1* and protein tyrosine phosphatase, non-receptor type 11 gene (*PTPN11*), resulting in combined phenotypes of Marfan syndrome (MIM:154700) and LEOPARD syndrome (MIM:151100) (5).

The co-existence of two Mendelian conditions in one individual brings challenges to the diagnosis and clinical management. The precise diagnosis of such conditions often relies on comprehensive genetic testing such as exome sequencing (ES) (6–8). Thus far, systematic investigations of dual molecular diagnosis have been performed in neurodevelopmental disorders (9, 10), genetic muscle diseases (11), endocrine dysfunction (10) and so on. However, the molecular epidemiology and phenotypic characteristics of dual diagnosis in skeletal abnormality is still elusive.

Here, we describe eight cases with dual molecular diagnosis from the Deciphering disorders Involving Scoliosis and COmorbidities (DISCO) study. We also report the phenotypic characteristics of these patients and clinical relevance for certain genes and Mendelian syndromes.

Results

Molecular epidemiology

In total, 1108 patients with skeletal abnormality from the DISCO study underwent ES. We identified eight probands with dual molecular diagnosis, including three (0.7%) from individuals with EOS, three (0.5%) from individuals with short stature, and two (2%) from individuals with CHFA (Table 1). All the eight probands have dual diagnosis of two autosomal dominant (AD) diseases. A total of 16 variants in 12 genes were identified. A substantial rate (5 of 10) of the identified causal variants were of *de novo* origin. The frequently observed molecular diagnoses (observed in more than one patient) include Osteogenesis Imperfecta Type I (*COL 1A1*, MIM:166200), Neurofibromatosis, Type I (*NF1*, MIM:162200) and Marfan Syndrome (*FBN1*, MIM:154700).

The complex clinical features of patients with dual molecular diagnosis

Patients with dual molecular diagnosis often present blended phenotypes of two diseases, which significantly complicate their diagnostic odysseys. Here we report the detailed clinical characteristics of these patients to demonstrate the effect dual molecular diagnosis on the diagnosis of skeletal abnormality.

Case 1

Patient DISCO-SCO2003P1972 was a 7-year-old boy with early-onset scoliosis (Figure 1.A, Supplementary Figure 1.A-B). At 2 years old, he was diagnosed with congenital dislocation of hip joint (Table 1) and underwent a surgical reduction. Meantime, a slight scoliosis was found during the hospitalization. At 7 years old, congenital scoliosis with segmentation failure of T10-L1 was identified through CT examination (Figure 1.A, Table 1). Physical examination showed disproportionate ocular hypertelorism and intellectual disability (Figure 1.A, Table 1). ES revealed a pathogenic heterozygous nonsense variant (c.2649G>A p.Trp883Ter) in *FBN1* (Table 1), which is associated with Marfan syndrome (MIM:154700) (3). Consistently, the proband also presented Marfan syndrome-related phenotypes such as mitral valve prolapse, mild arachnodactyly and scoliosis. This variant was inherited from his mother, who had severe scoliosis, arachnodactyly and long slender limbs (Figure 1.A). Through further analysis of the exome data, a *de novo* variant in *POGZ* (c.1180_1181del p.Met394ValfsTer9) (Table 1) was found in the proband. This variant was previously reported and was associated with White-Sutton syndrome (WHSUS) (MIM:616364) characterized by intellectual disability, ocular abnormalities and skeletal deformities (12), which largely overlapped with the phenotypes of this patient. Therefore, we suggested that the complex phenotypes of this patient could be explained by a combined effect of variants in *POGZ* and *FBN1*.

Case 2

In case 2, the proband (DISCO-SCO1908P0067) was first referred to the clinic due to scoliosis at the age of fifteen years. The patient was clinically diagnosed as adolescent idiopathic scoliosis (AIS) (Supplementary Figure 1.C), i.e., scoliosis with unknown etiology (Table 1). He also presented pectus carinatum (Table 1). He experienced fracture twice at the age of 10 and 13. ES revealed that he carried two pathogenic variants, including a *de novo* variant in *COL1A1* (c.1081C>T p.Arg361Ter) and a maternally inherited variant in *FBN1* (c.1453C>T p.Arg485Cys) (Table 1). These two reported variants led to dual molecular diagnosis consisting of osteogenesis imperfecta type I and Marfan syndrome(13, 14). Although reported patients with osteogenesis imperfecta type I often present short stature (15), the stature of this patient is relatively high tall (Height: 185cm) (Figure 1.B, Table 1), which might be related with his second diagnosis of Marfan syndrome. Although *FBN1* variant has been reported and considered as a pathogenic variant (14), no other features of Marfan Syndrome such as dolichostenomelia, arachnodactyly, joint laxity, velvety skin, ectopia lentis and cardiovascular manifestations were identified. This patient exemplified the apparently contrary effects of two monogenic disease on the same trait (height in this case).

Case 3

Patient DISCO-PCT2007P0019 was an 8-year-old girl. The proband suffered from right tibia fracture at the age of 2 years. At around the age of 6 years, the patient developed razorback deformity and unbalanced shoulder, accompanied with mild scoliosis. A progressive scoliosis was observed in the next 2 years (Supplementary Figure 1.D-E). A series of blended clinical phenotypes, such as blue sclera, cafe-au-Lait macules were observed as well (Figure 1.C, Table 1). We found vertebral malformations (T6, T7) in this patient (Figure 1.C) through radiographic examinations. Her father and younger brother presented with blue sclera but without any history of bone fracture. ES identified the one pathogenic variant in *NF1* (c.2307del p.Thr770LeufsTer21) and another pathogenic variant in *COL1A1* (c.2028+4A>G) (Table 1), both transmitted from her father. The *NF1* variant could lead to a frameshift of *NF1* (Table 1) and thus a loss-of-function effect, which is associated with neurofibromatosis type I and could explain the cafe-au-Lait macules in this patient (Figure 1.C, Table 1). The *COL1A1* splicing variant (c.2028+4A>G) has been previously described to cause osteogenesis imperfecta (16), which could explain the recurrent bone fracture history in this patient. Interestingly, both neurofibromatosis type I and osteogenesis imperfecta are associated with scoliosis but with incomplete penetrance (15, 17). Therefore, the scoliotic phenotype in this patient might be caused by the synergistic effects of the dual molecular diagnosis.

Case 4

In this case, we reported a 6-year-old boy (DISCO-WAX677) with short stature and craniofacial abnormalities including depressed nasal bridge and long philtrum (Table 1). Then the patient was detected to be double heterozygote for putatively pathogenic *ANKRD11* (c.2508dup p.Leu837ThrfsTer81) and *COL11A1* (c.1180_1181del p.Met394ValfsTer9) variants on WES (Table 1). The *ANKRD11* and *COL11A1* variants were confirmed as *de novo* and paternal status, separately (Table 1). The clinical findings of the present case are compared with the reported phenotypes of KBG syndrome (MIM:148050) and Marshall syndrome (MIM:154780), caused by *ANKRD11* and *COL11A1* variants, respectively. A comparison between the findings in the patient and reported phenotypes of KBG and Marshall syndrome indicated overlapping clinical features in this case.

Case 5

This patient (DISCO-AX168) was an 8-year-old girl with short stature and developmental delay. She also presented widespread cafe-au-Lait macules and lumbar scoliosis (Figure 1.D, Table 1). Additionally, her father (162cm) and mother (140 cm) were both presented with short stature. Brain magnetic resonance (MR) was performed and abnormality of the cerebral white matter was identified. According to previously study (18), combined with MR results and family history, the patient was diagnosed with idiopathic short stature (ISS). Subsequently, the girl was sequenced and two pathogenic variants (Table 1), including a splicing variant in the *NF1* gene (c.6705-1G>A) and a frameshift variant in the *GLI2* gene (c.1189del p.Val397CysfsTer124) were identified. Variants in *GLI2* have been shown to cause short stature, abnormal development of brain structures, hypopituitarism and facial dysmorphism in Culler-Jones syndrome (MIM:615849)(19). We suggested this patient's presentation represents a mixture of distinct phenotypes, i.e., Cafe-au-Lait spots for NF type 1 (*NF1*, MIM:162200) and short stature for Culler-Jones syndrome (MIM:615849).

Case 6

Case 6 (DISCO-AX282) was a 12-year-old girl with short stature (Table 1). She also presented mild scoliosis, low posterior hairline, hyper pigmentation and webbed neck (Figure 1.E, Table 1). ES was performed to find the potential molecular etiology. A pathogenic nonsense variant in *TP63* (c.109C>T p.Arg37Ter) and another pathogenic missense variant in *PTPN11* (c.1510A>G p.Met504Val) were identified (Table 1). On the basis of published studies (20, 21), as well as phenotypes in this patient, we concluded this patient carried variants in both *TP63* and *PTPN11*, resulting in a blended phenotype of Rapp-Hodgkin syndrome (MIM: 129400) and Noonan syndrome (MIM: 163950). Overlapping phenotypes (especially craniofacial malformations) of these two syndromes make the diagnosis challenging if solely based on clinical features.

Case 7

A 3-year-old proband (DISCO-RDD2001P0005) presented at birth with widespread interphalangeal joint contractures of hand (Figure 1.F) and atrial septal defect (Table 1). His father also experienced hand contracture deformities during his childhood. ES revealed a heterozygous variant (c.3437A>G p.Tyr1146Cys) in *FBN2* gene and a heterozygous *ANKRD11* variant (c.3024_3025del p.Lys1009GlyfsTer8), both with unknown origin (Table 1). The *FBN2* variant could lead to Beals syndrome (Congenital contractual arachnodactyly) (MIM:121050), which is characterized by hand interphalangeal joint contractures (22, 23); The *ANKRD11* mutation could cause KBG syndrome, which could explain the atrial septal defect in this patient (24). Therefore, this patient present mixed phenotypes of two distinct Mendelian disorders.

Case 8

This case is a 5-year-old male (DISCO-P19009402) with complex phenotypes. He presented congenital syndactyly of hand and foot (Supplementary Figure 1.F-G), cloverleaf skull, orbital hypertelorism, proptosis and midfacial hypoplasia (Table 1). He also had a history of hyperthermia during general anesthesia. ES identified a *de novo* missense variant (c.755C>G p.Ser252Trp) in *FGFR2* gene and a paternally inherited frameshift variant in *RYR1* gene (c.12788_12793dup p.Glu4263_Gly4264dup) (Table 1). The *FGFR2* variant occurred in a known Apert syndrome hotspot (25). As reported, Apert syndrome was characterized by craniosynostosis, proptosis, midfacial hypoplasia and severe syndactyly of the hands and feet (25), which are concordant with the phenotypes of this patient. Pathogenic *RYR1* variants are associated with malignant hyperthermia susceptibility 1 (MHS 1) (MIM:145600) (26), which could explain the hyperthermia history in the patient.

Table 1

Summary of the clinical and molecular findings of studied subjects

Discussion

The development of ES has significantly improved diagnostic yield of rare disease in recent years. Through analyzing the ES data from 7374 patients, 2182 independent molecular diagnoses were reported by Posey et al. (6, 8). A molecular diagnosis involving a mendelian disease was reported for 2076 patients (28.2%) and two or more molecular diagnoses were reported for 101 patients (4.9%) (6, 8). Additionally, copy number variants (CNVs) and single nucleotide variants (SNVs) as a part of multiple diagnoses was reported in 12 of 101 patients (11.9%) by Posey et al (6). Yang et al. found ES identified the underlying genetic defect in 25% of consecutive patients referred for evaluation of a possible genetic condition and 4.6% patients with blended phenotypes resulting from two single gene defects (7).

In our study, most probands (5 out of 8) had family members for at least one of the diseases, consistent with the report by Balci et al (27). All the 8 probands have variants in AD disease genes and *de novo* variants were the most common pathogenic variants origin and accounted for 40% of pathogenic variants in 8 families. These results are consistent with previously published articles, which revealed that AD was the most frequently seen inheritance mode among patients with multiple diagnoses (8). There're two types of effect caused by the mutual interaction of two pathogenic variants. One was called "synergistic effect", which means that the combination of two mutational genes in patients would lead to more severe phenotypes. For instance, Xe et al. revealed a patient with one variant in *CSNK2A1* and another in *TRPS1*, which resulted in a dual molecular diagnosis of tricho-rhino-phalangeal syndrome type I (TRPS I) and Okur-Chung neurodevelopmental syndrome (OCNDS). These two syndromes are both associated with short stature. Notably, compared with other patients with single diagnosis as well as his parents, this patient has a more severe degree of short stature (9). Moreover, Ye et al. reported a familial case with 13 patients affected by osteogenesis imperfecta (OI) type I, short stature and advanced bone age, with or without early-onset osteoarthritis and/or osteochondritis dissecans (SSOAO). The proband was found to have two variants in two genes: *COL1A1* and *ACAN*. After comprehensive analysis of the height within the family who carried either or both of the variants, this study discovered a synergistic effect that the patients with two variants present the most severe form of short stature (28). These manifestations resembled clinical presentations of one of our patients (DISCO-SCO2003P1972). The kid was presented with a segmentation failure of T10-L1 of spinal vertebrae, which was extremely unusual phenotype of classical Marfan Syndrome. Additionally, a severe scoliosis, which presented with a thoracolumbar curve (Cobb > 80°), were observed in the kid. We concluded that the *POGZ* and *FBN1* variants both contributed to the skeletal abnormality in this patient. However, in some patients who were diagnosed with dual molecular diagnosis, certain phenotypes may be opposite to that caused by a single genetic mutation. We proposed that these phenomena were caused by an antagonistic effect. Tang et al. reported a patient with variants in both *FBN1* and *PTPN11* genes with combined phenotypes of Marfan (MIM:154700) and LEOPARD (MIM:151100) syndromes (5). However, this patient presented with short stature, other than classical tall stature of Marfan syndrome. We suppose that this phenomenon probably caused

Case Number	Case ID	Age	Sex	Inheritance	Gene	Clinical diagnosis	Molecular diagnosis	Zygoty	Transcript numbers	Origin	Variant type
Case 1	SCO2003P1972	7	M	AD	<i>POGZ</i>	CS II	White-Sutton Syndrome	Het	NM_015100.3	<i>de novo</i>	Frank
Case 1	SCO2003P1972	7	M	AD	<i>FBN1</i>	CS II	Marfan Syndrome	Het	NM_000138.4	maternal	Non-
Case 2	SCO1908P0067	18	M	AD	<i>COL1A1</i>	AIS	Osteogenesis imperfecta	Het	NM_000088.3	<i>de novo</i>	Stop
Case 2	SCO1908P0067	18	M	AD	<i>FBN1</i>	AIS	Marfan Syndrome	Het	NM_000138.4	maternal	Miss
Case 3	PCT2007P0019	8	F	AD	<i>NF1</i>	NFS	Neurofibromatosis, Type 1	Het	NM_000267.3	paternal	Frank
Case 3	PCT2007P0019	8	F	AD	<i>COL1A1</i>	NFS	Osteogenesis imperfecta	Het	NM_000088.3	paternal	Splic
Case 4	WAX677	6	M	AD	<i>ANKRD11</i>	GHD	KBG syndrome	Het	NM_013275.5	<i>de novo</i>	Non-
Case 4	WAX677	6	M	AD	<i>COL11A1</i>	GHD	Marshall syndrome	Het	NM_001854.3	paternal	Frank
Case 5	AX168	8	F	AD	<i>NF1</i>	ISS	Neurofibromatosis, Type 1	Het	NM_000267.3	NA	Splic
Case 5	AX168	8	F	AD	<i>GLI2</i>	ISS	Culler-Jones syndrome	Het	NM_005270.4	NA	Frank
Case 6	AX282	12	F	AD	<i>TP63</i>	ISS	Rapp-Hodgkin Syndrome	Het	NM_003722.4	NA	Non-
Case 6	AX282	12	F	AD	<i>PTPN11</i>	ISS	Noonan Syndrome	Het	NM_002834.3	NA	Miss
Case 7	RDD2001P0005	2	M	AD	<i>FBN2</i>	CPC	Beals Syndrome	Het	NM_001999.3	paternal	Miss
Case 7	RDD2001P0005	2	M	AD	<i>ANKRD11</i>	CPC	KBG Syndrome	Het	NM_013275.5	<i>de novo</i>	Frank
Case 8	P19009402	4	M	AD	<i>FGFR2</i>	Syndactyly	Apert Syndrome	Het	NM_000141.4	<i>de novo</i>	Miss
Case 8	P19009402	4	M	AD	<i>RYR1</i>	Syndactyly	Malignant Hyperthermia Susceptibility 1	Het	NM_000540.2	paternal	Frank

by the predominant influence of the *PTPN11* mutation over the *FBN1* mutation (5).

All the dual diagnosis condition in our study were identified through ES. However, the high expense of ES hinders it from being either a stand-alone or a first-tier diagnostic approach. Therefore, selection the most appropriate molecular diagnostic tool is important when ordering genetic testing. Single-gene tests should be recommended when the clinical features for a patient were typical for a specific disorder and the association between the disorder and a single gene was well-established (29). For example, *TBX6*-associated congenital scoliosis was characterized by simple hemi-/wedge-shaped vertebrae in the lower half of the spine (30–32). Furthermore, our previous study found that a novel *de novo FBN1* variant could explain the Marfanoid–progeroid–lipodystrophy syndrome (MIM:616914) (33). Under these circumstances, single-gene testing will be more preferred. In contrast, in cases with complex phenotypes as shown in our examples, ES can be utilized as first line test which could shorten the diagnostic odyssey of the patients (29).

In conclusion, this study revealed the molecular epidemiology and complex diagnostic odyssey of dual molecular diagnosis through analyzing the clinical traits of bone abnormality in eight patients.

Conclusions

This study recruited eight patients with dual molecular diagnosis from DISCO study and described the clinical traits of skeletal abnormalities. We reported the molecular epidemiology and complex diagnostic odyssey of dual molecular diagnosis.

Methods

Study design

This is a retrospective study which reports the clinical and genetic characteristics of a group of patients with dual molecular diagnosis.

Subjects

Cases with skeletal abnormality from the DISCO study (<http://www.discostudy.org/>) who underwent ES were included. The types of skeletal abnormality include early-onset scoliosis (EOS) (N =447), short stature (N=561), and congenital hand/foot anomaly (CHFA) (N=100). Deep phenotyping and radiological examinations including X-ray, computed tomography (CT), and magnetic resonance imaging (MRI) were performed on each patient as previously reported (3, 34), (Fan et al., *Journal of Genetics and Genomics*, 2021, in press). Written informed consent was obtained from every participant; if the participant was younger than 16 years old, written informed consent was obtained from their parents or legal guardians. The study was approved by the institutional review board of PUMCH (JS-2364), Jishuitan Hospital (201808-09) and the Second Affiliated Hospital of Guangxi Medical University (G-1-1).

Exome sequencing and variant interpretation

ES was performed on DNA extracted from blood of all 8 probands and their family members. The sequencing data were analyzed and annotated using an in-house developed analytical pipeline, Peking Union Medical College Hospital Pipeline (PUMP) (35-37). All variants were presumed to be pathogenic were subjected to Sanger sequencing.

Identification of dual molecular diagnosis

Patients with more than one molecular diagnosis from the included patients were selected for analyses. Each molecular diagnosis was manually curated based on the pathogenicity of the variants and the Mendelian expectations for inheritance mode. The pathogenicity of the variants was evaluated according to the American College of Medical Genetics and Genomics (ACMG) guidelines (38). The Mendelian expectations for inheritance mode include autosomal dominant (AD) inheritance, autosomal recessive (AR) inheritance and X-linked dominant/recessive (XLD/XLR) inheritance. For AD/XLD traits, one heterozygous pathogenic/likely pathogenic variant is sufficient to establish a molecular diagnosis. For AR/XLR traits, one homozygous, one hemizygous or one pair of compound heterozygous pathogenic/likely pathogenic variants are required for a molecular diagnosis.

Abbreviations

COL1A1: Collagen, Type I, Alpha-1; *NF1*: Neurofibromatosis, Type I; *FBN1*: Fibrillin-1; *FBN2*: Fibrillin-2; *PTPN11*: protein tyrosine phosphatase, non-receptor type 11 gene; ES: exome sequencing; EOS: early-onset scoliosis; *WHSUS*: White-Sutton syndrome; *Gli2*: GLI-Kruppel family member 2; *ANKRD11*: Ankyrin repeat domain-containing protein 11; *FGFR2*: Fibroblast growth factor receptor 2; *RYR1*: Ryanodine receptor 1; *CSNK2A1*: Casein kinase II, alpha subunit; *TRPS1*: trichorhinophalangeal syndrome type I.

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board of PUMCH (JS-2364), Jishuitan Hospital (201808-09) and the Second Affiliated Hospital of Guangxi Medical University (G-1-1). All participants gave informed consent.

Consent for publication

Consent for publication was obtained from all participants.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NW directed the project. NW, JZ and GQ designed the study. LL, LS and YC were involved in data management and statistical analysis and wrote the first draft of the manuscript. CY, YH, HD and SC gathered detailed clinical information for the study. XF, WT and SZ analyzed the data. ZW, GQ, JZ and NW supervised the data analysis, and reviewed and commented on all drafts. All other authors coordinated the study and critically revised the manuscript. The author(s) read and approved the final manuscript.

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Figures

Figure 1

Representative clinical photographs of 6 patients with skeletal abnormality. A Patient DISCO-SCO2003P1972. B Patient DISCO-SCO1908P0067. C Patient DISCO-PCT2007P0019. D Patient DISCO-AX168. E Patient DISCO-AX282. F Patient DISCO-RDD2001P0005

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