Early onset juvenile SLE associated with a novel mutation in protein kinase C delta

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Abbreviations: ANA, anti-nuclear antibody; CRP, C-reactive protein; dbSNP, single nucleotide polymorphism database; DNA, deoxyribonucleic acid; ESR, erythrocyte sedimentation rate; Gly, glycine; IgG, immunoglobulin G; IVIG, intravenous immunoglobulin; jSLE, juvenile systemic lupus erythematosus; PKCδ, protein kinase C delta; *PRKCD*, Protein Kinase C Delta gene; Ser, serine; SLE, systemic lupus erythematosus; Trp, tryptophan; WES, whole-exome sequencing.

Contributors' statement

Dr Nanthapisal designed the study, carried out the analyses, drafted the initial manuscript, and approved the final manuscript as submitted

Dr Omoyinmi, Miss Murphy and Dr Standing coordinated and supervised data analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Dr Eisenhut coordinated data collection, reviewed the manuscript, and approved the final manuscript as submitted.

Dr Eleftheriou and Prof Brogan conceptualized and designed the study, critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ABSTRACT

Juvenile systemic lupus erythematosus (jSLE) is rare before 5 years-of-age. Monogenic causes are suspected in cases of very early onset jSLE particularly in the context of a family history and/or consanguinity. We performed whole-exome sequencing and homozygosity mapping in the siblings presented with early-onset jSLE. A novel homozygous missense mutation in Protein Kinase C Delta (c.1294G>T; p.Gly432Trp) was identified in both patients. One patient showed a marked clinical response and resolution inflammation with rituximab therapy. This report demonstrates the clinical importance of identifying monogenic causes of rare disease to provide a definitive diagnosis, help rationalize treatment, and to facilitate genetic counseling.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex, severe, chronic, and sometimes life-threatening disease. Many factors contribute to the development of juvenile SLE (jSLE) including genetics, immune dysfunction and environmental factors[1]. jSLE is rare before 5 years-of-age, and where this occurs monogenic causes should be considered, particularly if there is a family history of SLE, and/or consanguinity. There is now an ever-expanding list of monogenic causes of SLE (Table 1), and many present very early in life[2-6]. We describe two siblings who presented with early-onset jSLE in whom we identified a homozygous missense mutation in the Protein Kinase C Delta (*PRKCD*) gene. This report demonstrates the clinical importance of identifying monogenic causes of rare disease to provide a rapid and definitive diagnosis, help rationalize treatment, and to facilitate genetic counseling.

CASE

The index case presented at the age of 12-months with scarring alopecia, rash affecting the scalp, and a photosensitive malar rash (figure 1A). She also had hepatosplenomegaly and bruising of the skin (Figure 1B) and erythematous, non-pruritic, vasculitic rash affecting the hands and feet (Figure 1C and D). Oral mucosa was normal. Other features were mild monoarthritis of the knee for more than 2 months (clinically not typical of septic arthritis, and hence joint not aspirated), and an episode of acute onset of fever, epistaxis, rectal bleeding and pancytopenia (haemoglobin 10 g/L, white blood cells 3,060 cells/mL, neutrophil count 1,540 cells/mL, lymphocyte count 1,010 cells/mL, platelet count 87,200 cells/mL) at the age of 3 years. Further investigations showed elevated inflammatory markers (elevated ESR 125 mm/hour and CRP 30 mg/L), positive anti-nuclear antibody 1:320, anti-double stranded DNA at 37 (normal < 10) and positive anti-extractable nuclear antigens (ribonucleoprotein, Smith

and ribosomal P protein). Complement assays were normal: C1q 42 mg/L (normal range 50-250 mg/L), C3 0.66 g/L (normal range 0.75-1.65 g/L), C4 0.1 g/L (normal range 0.14-0.54 g/L), functional classical complement pathway assay activity was 75% (normal > 40%), and functional alternative complement pathway assay activity was 99% (normal > 10%). Renal function and transaminase levels were entirely normal.

The pedigree of the family is shown in figure 2A. She was the second child of consanguineous parents (first cousins once removed, i.e. the father was the child of the mother's first cousin) originally from Pakistan. The 5-year-old sister of the index case had identical symptoms and signs, which started at the age of 12-months. Her parents were initially healthy, although the mother subsequently developed SLE during her third pregnancy. This manifested as mild cutaneous malar rash, and arthralgia; but no evidence of pancytopenia or other organ involvement.

Both siblings were diagnosed with familial SLE, with fulfillment of 6 out of 11 of the American College of Rheumatology classification criteria: malar rash, photosensitivity, arthritis, haematological disorder, immunologic disorder and positive ANA[7]. Both patients were initially treated in the first instance with: pulse methylprednisolone (30 mg/kg for 3 days followed by oral prednisolone 2 mg/kg/day), hydroxychloroquine (5 mg/kg/day) and azathioprine (2 mg/kg/day). Despite this, over the next 6 months both children progressively deteriorated with pancytopenia with a platelet count in the range of 60,000 – 84,000 cells/mL. Both siblings demonstrated only a transient response to further pulses of intravenous methylprednisolone and intravenous immunoglobulin (IVIG; 2g/kg, for persistent thrombocytopenia). Therefore, rituximab (750 mg/m^2, repeated two weeks later) was given to the index case, which resulted

in a rapid and sustained (13 months at the time of writing) clinical improvement, with complete normalisation of the full blood count, and normalisation of inflammatory markers. Rituximab (at the same dose) was then given to the older sibling, who unfortunately developed anaphylaxis during the second infusion, although still B-cell depleted successfully, and remains in clinical remission 12 months later.

Work up for suspected monogenic SLE

All experimental work was performed with ethical approval (ethics number: 08H071382) and with written informed consent from all participants. Both patients were screened using conventional Sanger sequencing for known monogenic causes of SLE for: *TREX1, SAMHD1, C1qA, C1qB,* and *C1qC*, all of which were negative (wild-type). Homozygosity mapping was therefore performed in both patients and both parents (see Supplementary Methods). Whole-exome sequencing (WES) was subsequently performed only in the index case (see Supplementary Methods). These studies revealed a homozygous missense mutation (c.1294G>T; p.Gly432Trp) in the *PRKCD* in the index case (see Supplementary Results for further details), subsequently confirmed using Sanger sequencing. The c.1294G>T substitution is a novel mutation not yet annotated in the dbSNP, the ClinSeq database[8], 6500ESP[9] or the 1000 genomes project databases[10]. This homozygous c.1294G>T mutation was also confirmed in her affected sister using Sanger sequencing (figure 2B). Her currently asymptomatic 1-year-old brother was also homozygous for the same mutation. As expected, both parents were confirmed to be heterozygous carriers of the same mutation.

DISCUSSION

PRKCD is located on chromosome 3p21.31. It encodes protein kinase C delta (PKCδ), a member of serine/threonine kinase family that plays a role in apoptosis and proliferation of cells[11]. PKCδ is also known to play a role in B-cell negative selection [12] and has been shown to prevent proliferation of B and T cells in response to stimulation in the mouse model[13]. PKCδ deficient mice were found to have features of SLE including anti-double strand DNA autoantibodies, glomerulonephritis with IgG containing immune-complex.deposition and lymphocyte infiltration in multiple organs[13, 14].

The p.Gly432Trp mutation affects the protein kinase activity domain which is located between amino acid 349 and 603[15]. Thus, this mutation is likely to cause a loss of function of the kinase activity of PKCδ. The p.Gly432Trp mutation is predicted to be deleterious in pathogenicity prediction algorithms (supplemental Table S-3). One limitation of our study was that we did not perform any functional experiments to assess protein expression or function, or detailed B cell immunotyping (for various practical reasons, including limited access to clinical samples). We suggest however, that the clinical features are explained by the mutations we identified in *PRKCD*, particularly since WES did not identify any other plausible genetic cause.

Mutation of *PRKCD* (p.Gly510Ser) has previously been identified as the cause of early-onset jSLE by Belot *et al* in 3 children of consanguineous unions. Mutations identified were shown to cause reduction of PKCδ expression and phosphorylation activity, and transfected lymphoblastoid cell lines were found to be resistant to apoptosis, reversible by co-expression of non-mutant protein. In the Belot study, primary B-cells from patients and heterozygous carriers also exhibited a higher proliferation rate than wild-type individuals following

stimulation of the B-cell receptor, CD40 and toll-like receptor 9 compared with wild-type B-cells[16].

Rituximab, a monoclonal antibody against CD20 present on pre-B and mature B lymphocytes, was (fortuitously) the optimal therapeutic drug of choice in these patients because of the possible immunological defects in B cells caused by the mutation in *PRKCD*. Since one sibling developed anaphylaxis to rituximab, other B-cell targeted therapies such as ofatumumab (an alternative fully-humanised monoclonal antibody against CD20)[17], or belimumab (a fully-humanized monoclonal antibody against B-lymphocyte stimulator B-lyS)[18] are being considered for future treatment[19]. Alternatively, it is possible that hematopoietic stem cell transplantation may ultimately be required, although to the best of our knowledge has not yet been performed in SLE caused by mutations in *PRKCD* (Belot A, personal communication).

Interestingly the mother, who is a heterozygous carrier of the mutation, developed SLE during pregnancy with her third child. The combined contribution of heterozygous carriage and hormonal changes occurring during pregnancy may be responsible for the new-onset of SLE during pregnancy[20]. The youngest brother aged eighteen months at the time of writing also has the homozygous mutation and is thus far asymptomatic but will be closely monitored for the development of symptoms.

CONCLUSIONS

We have described a rare monogenic form of jSLE caused by a novel but very likely damaging homozygous mutation affecting the active region of *PRKCD*. Identification of this additional disease causing variant of *PRKCD* provides further supportive evidence for this gene to be included in routine genetic screening for suspected monogenic SLE. Securing this molecular diagnosis not only provided us with a definitive diagnosis, but also explained the dramatic and complete therapeutic response to B-cell depletion (despite failing other therapies), and will direct the choices made for future treatment options. Since the list of monogenic causes of SLE is increasing (Table 1), next-generation sequencing offers the opportunity to screen all the known genetic causes rapidly, and for a fraction of the cost of conventional sequencing, and should be considered in all cases of early-onset (<5-years) jSLE, particularly for consanguineous families.

Table 1: Monogenic causes of systemic lupus erythematosus

Gene	Locus	Inheritance	Clinical features	Onset of SLE/SLE-like (years)	SLE/ SLE-like features	Specific treatment*	References
Complement Cascade							
C1QA	1p.36.12	AR	SLE, ICD, RI	1-40	Mucocutaneous and renal involvement, positive ANA, Anti-dsDNA, ENA antibodies, increased cardiovascular risk	Fresh frozen plasma, HSCT	6, 21-23
C1QB	1p36.12	AR	SLE, ICD, RI				
C1QC	1p.36.11	AR	SLE, ICD, RI				
C1R	12p13	AR	SLE, ICD, RI	<1	Mucocutaneous ,neurological and renal involvement	-	24
C1S	12p13	AR	SLE, ICD, RI	<1	Mucocutaneous and renal involvement, autoimmune thyroiditis, autoimmune hepatitis	-	25, 26
C2	6p21.3	AR	SLE, RI, UCTD, vasculitis, Sjogren's syndrome	>10	Mucocutaneous, haematological and renal involvement, arthritis	-	1, 27-29
C4	6p21.3	AR	SLE, ICD, RI, Rheumatoid arthritis	2-40	Mucocutaneous, haematological and renal involvement, vasculitis	-	30
TREX1	3p21.31	AD	SLE, FCL, RVCL	4-adulthood	Cold-induced chilblain lupus, photosensitive rash, haematological and neurological involvement, arthralgia/arthritis	-	31
		AR	AGS1	<1	Cold-induced chilblain lupus	-	32, 33
RNASAH2A	19p13	AR	AGS4	<1	Cold-induced chilblain lupus	-	32, 33

RNASAH2B	13q14	AR	AGS2	<1	Cold-induced chilblain lupus	-	32, 33
RNASAH2C	11q13	AR	AGS3	<1	Cold-induced chilblain lupus	-	32, 33
SAMHD1	20q11	AR	AGS5	<1	Cold-induced chilblain lupus	-	32, 33
		AD	FCL		Cold-induced chilblain lupus	-	34
ADAR	1q21.3	AR	AGS6	<1	Cold-induced chilblain lupus	-	35
DNASE1	16p13.3	AD	Sporadic SLE	9-13	Systemic lupus, Sjogren's syndrome, high ANA	-	5
DNASE1L3	3p14.3	AR	Familial SLE	2-12	Mucocutaneous and renal involvement, positive ANCA and anti-cardiolipin antibodies	-	36
PRKCD	3p21.31	AR	Familial SLE	<5	Cutaneous vasculitis, haematological involvement, positive ANA and dsDNA antibodies	-	16
ACP5	19p13.2	AR	SPENCD, skeletal dysplasia, delayed development, intracranial calcification, immune dysregulation	<1-15	Haematological and renal involvement, positive ANA	-	37
SLC7A7	14q11.2		Lysinuric protein intolerance with some cases of SLE	>10	Renal involvement, vasculitis, haemophagocytic lymphohistiocytosis	-	38, 39
IFIH1	2q24.2	AD	SLE with IgA deficiency, RI, limb spasticity	6-12	Arthritis, cutaneous vasculitis, haematological involvement, positive ANA and dsDNA antibodies, secondary antiphospholipid syndrome	-	4
TMEM173	5q31.2	AD	Familial SLE	2-20	Arthritis, cutaneous vasculitis, haematological and pulmonary involvement, positive ANA	-	40

AD: autosomal dominant, AGS: Aicardi-Goutières Syndrome, ANA: anti-nuclear antibody, ANCA: anti-neutrophil cytoplasmic antibody, AR: autosomal recessive, dsDNA: double stranded DND, ENA: extractable nuclear antigen, FCL: Familial Chilblain lupus, HSCT: haematopoietic stem cells transplantation, ICD: immune complex disease, RI: recurrent infection, RVCL: Retinal Vasculopathy with Cerebral Leukodystrophy, SLE: systemic lupus erythematosus, SPENCD: Spondyloenchondrodysplasia, UCTD: undifferentiated connective tissue disease

*all reported treatments described are case reports

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Figure 1 A: A 3-year-old with early-onset juvenile systemic lupus erythematosus caused by homozygous mutation of *PRKCD* manifesting with alopecia and rash affecting the scalp and face, with sparing of the nasolabial folds. Figure B: hepatosplenomegaly, and bruising from thrombocytopenia; Figure C & D: erythematous, non-pruritic, vasculitic rash affecting the palms and soles.

Figure 2 A*:* Pedigree of the family**.** The index case is V**-**2; the affected sister is V**-**1**.** B*:* Sanger sequencing chromatogram of *PRKCD* at position c.1294 *(*in the red-dashed box*)*. The reference base in wild type is G shown above the chromatogram. The chromatogram shows a heterozygous pattern with two peaks of T and G in parents, and a homozygous T in patient, affected sister and unaffected brother. *(*T is red and G is black*)*.