Emerging phenotypes linked to variants in SAMD9 and MIRAGE syndrome

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Background: Heterozygous de novo variants in SAMD9 cause MIRAGE syndrome, a complex multisystem disorder involving Myelodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes, and Enteropathy. The range of additional clinical associations is expanding and includes disrupted placental development, poor post-natal growth and endocrine features. Increasingly, milder phenotypic features such as hypospadias in small for gestational age (SGA) boys and normal adrenal function are reported. Some children present with isolated myelodysplastic syndrome (MDS/monosomy 7) without MIRAGE features.

Objective: We aimed to investigate: 1) the range of reported SAMD9 variants, clinical features, and possible genotype-phenotype correlations; 2) whether SAMD9 disruption affects placental function and leads to pregnancy loss/recurrent miscarriage (RM); 3) and if pathogenic variants are associated with isolated fetal growth restriction (FGR).

Methods: Published data were analyzed, particularly reviewing position/type of variant, pregnancy, growth data, and associated endocrine features. Genetic analysis of SAMD9 was performed in products of conception (POC, n=26), RM couples, (couples n=48; individuals n=96), children with FGR (n=44), SGA (n=20), and clinical Silver-Russell Syndrome (SRS, n=8), (total n=194).

Results: To date, SAMD9 variants are reported in 116 individuals [MDS/monosomy 7, 64 (55.2%); MIRAGE, 52 (44.8%)]. Children with MIRAGE features are increasingly reported without an adrenal phenotype (11/52, 21.2%). Infants without adrenal dysfunction were heavier at birth (median 1515 g versus 1020 g; P < 0.05) and born later (median 34.5 weeks versus 31.0; P < 0.05) compared to those with adrenal insufficiency. In MIRAGE patients, hypospadias is a common feature. Additional endocrinopathies include hypothyroidism, hypo- and hyper-glycemia, short stature and panhypopituitarism. Despite this increasing range of phenotypes, genetic
Introduction

SAMD9 (sterile α motif domain-containing protein 9) is a single exon gene that encodes a 1,589-amino acid protein and is located on the long arm of chromosome 7 (7q21.2) in humans, next to its paralogous gene SAMD9L (SAMD9-like) in a head-to-toe orientation (1). The molecular role of SAMD9 is not fully elucidated, however it has been shown to be a cytoplasmic protein involved in viral host defense mechanisms, cell proliferation, endosomal fusion and tumor suppression (2–6).

Deleterious loss-of-function variants in SAMD9 were originally reported to be associated with normophosphatemic familial tumoral calcinosis in rare cases (OMIM: 610455) (7, 8). However, it is now well established that heterozygous gain-of-function variants in SAMD9 can be identified in children with a complex multisystem condition named MIRAGE syndrome (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital (gonadal) phenotypes, and enteropathy) (OMIM: 617053) (9–11). Although this acronym embraces several common features, the range of additional clinical associations is expanding and includes achalasia/gastroesophageal reflux, recurrent intussusception, renal features (focal segmental glomerulus sclerosis), dysautonomia, autoinflammation, learning difficulties, and hypocalcemia/corneal anesthesia, amongst other findings (12–21). Disrupted placental development has also been reported, as well as poor post-natal growth (22).

The missense gain-of-function variants causing MIRAGE syndrome result in decreased cell proliferation and growth restriction in in vitro model systems, highlighting the innate role of SAMD9 as a growth repressor. Most SAMD9 variants occur de novo, however SAMD9 patients can inherit variants from asymptomatic parents either due to germline variants or revertant mosaicism events (20, 23). Indeed, dynamic, somatic revertant mechanisms are now established as frequent genomic events in individuals with pathogenic SAMD9 variants and are often seen in the hematopoietic system/bone marrow. Here, cells with secondary changes that “remove” the primary growth repressive SAMD9 variant have a clonal growth advantage. These changes include the progressive development of monosomy 7 or deletion of 7q (containing SAMD9); secondary loss-of-function variants in SAMD9 (nonsense, frameshift, missense); or acquired uniparental disomy of the “wild-type” allele (9, 10). Secondary events usually occur in cis to ameliorate the effects of the primary gain-of-function variants in the proband and, at least in the hematopoietic lineage, serve to modify disease phenotype. However, when monosomy 7 occurs there is a risk of myelodysplastic syndrome due to loss of SAMD9, SAMD9L, GATA2 and other factors, and an additional risk of leukemia if further somatic changes occur.

To date, more than 50 patients have been reported with severe growth restriction phenotypes due to gain-of-function changes in SAMD9 (9, 10, 12–22, 24–38). Although endocrine features such as primary adrenal insufficiency and gonadal dysgenesis were originally a core part of the syndrome, it is emerging that these features may be more variable. For example, SAMD9 pathogenic variants have now been identified in individuals with hypoplasias (46,XY DSD) and born small for gestational age (SGA), and increasingly in children without primary adrenal insufficiency (22). Other endocrine systems may be affected, such as the thyroid gland. Thus, the endocrine phenotype of MIRAGE syndrome is likely to be more variable than originally described and may comprise additional features not always reported.

We hypothesize that: 1) different SAMD9 variants, or variants in different domains of the protein, are associated
with milder or diverse endocrine phenotypes, with a focus on adrenal insufficiency; 2) severe gain-of-function of SAMD9 may affect placental function and lead to pregnancy loss and recurrent miscarriage (RM); and that 3) pathogenic variants could be found in children with fetal growth restriction (FGR) as the predominant feature. Determining whether SAMD9 variants cause isolated growth restriction is important as these children could develop monosomy 7, endocrinopathies or immune dysfunction, and could require personalized management such as bone marrow/stem cell transplantation.

Materials and methods

Meta-analysis of SAMD9-associated variants/MIRAGE syndrome

A systematic review was undertaken to identify published reports of individuals with SAMD9-associated conditions in the literature. PubMed was searched using the term “SAMD9”, up to May 2022. For this review, a particular focus was put on position and type of variant, pregnancy and growth data, and reported associated endocrine features. All published SAMD9 variant-carrying individuals included in our study and their related publication link are available here http://doi.org/10.17605/OSF.IO/WMVXY (39).

Study cohort and ethics

Samples were provided by the Wellbeing of Women Baby Bio Bank (BBB) (http://www.ucl.ac.uk/babybiobank) supported by University College London, Imperial College London and Wellbeing of Women (40) and the Moore Cohort with ethical approval (BBB Research Ethics Committee references: 09/H0405/30 and 09/H0405/30+5; Moore cohort reference: 2001/6029). Five different cohorts were included (Table 1): 1) Products of conception (POC) (n=26), spontaneous loss of pregnancy between 9-11 weeks gestation; 2) Recurrent miscarriage (RM) (n=96; 48 couples), couples who experienced three or more consecutive loss of pregnancies before 20 weeks gestation; 3) FGR (n=44), a fetus with growth restriction of unknown etiology in utero (41) and in this cohort having a weight less than 3rd centile (42); 4) Small for gestational age (SGA) (n=20), a baby with birth weight below the 10th percentile for gestational age (42); 5) Clinical Silver-Russell Syndrome (SRS) (n=8), based on Consensus guidelines for diagnosis (43) and all double negative for H19 hypomethylation and maternal UPD7.

DNA extraction and genetic analysis

DNA was isolated from blood leukocytes and POC as previously described (40, 44, 45). DNA samples were subjected to genetic analysis using the next generation sequencing (NGS) methodologies outlined below.

Whole exome sequencing

SGA samples underwent whole exome sequencing (WES) using the Agilent SureSelect Human All Exon V5 kit (Agilent Technologies Inc., Santa Clara, USA) by BGI (BGI Genomics, Hong Kong, China), and were sequenced with the high-throughput sequencing platform of Complete Genomics (Complete Genomics Inc., San Jose, USA) as detailed in Stalman et al. (44).

HaloPlex targeted NGS panel

A HaloPlex DNA targeted gene enrichment panel was designed using SureDesign software (Agilent Technologies Inc., Santa Clara, USA) to capture known and candidate genes involved in fetal growth restriction, including SAMD9. A detailed protocol has been described previously (45). In brief, FGR and POC samples were sequenced on a NextSeq sequencer

| Cohort                     | Number of Individuals | Description                                                                 | Sequencing Panel                        |
|----------------------------|-----------------------|-----------------------------------------------------------------------------|------------------------------------------|
| Product of Conception (POC)| 26                    | DNA from spontaneous loss of pregnancies between 9-11 weeks gestation       | HaloPlex targeted NGS                   |
| Recurrent Miscarriage (RM) | 96                    | DNA from couples who experienced 3 or more consecutive loss of pregnancies before 20 weeks gestation | HaloPlex targeted NGS                   |
| Fetal Growth Restriction (FGR)| 44              | DNA from newborns with a birth weight less than the 3rd percentile         | HaloPlex targeted NGS (n=35) and Nonacus Cell3TM Target NGS panel (n=9) |
| Small for Gestational Age (SGA)| 20              | DNA from newborns with a birth weight below the 10th percentile              | Agilent SureSelect Human All Exon V5 WES |
| Silver-Russell Syndrome (SRS)| 8                  | DNA from children with sporadic clinical Silver-Russell Syndrome            | Nonacus Cell3TM Target NGS panel        |

Description of individual cohorts analyzed in our study, showing the total number of cases in each group and the sequencing panels used.

NGS, next-generation sequencing; WES, whole exome sequencing.
Nonacus targeted NGS panel

A Nonacus Cell3TM target NGS panel (Nonacus Ltd., Birmingham, UK) was designed to cover the coding regions of \textit{SAMD9}. Nine samples from children with FGR and eight with SRS were sequenced on a MiSeq platform (Illumina Inc., San Diego, USA). Binary alignment map (BAM) files were produced by aligning FASTQ files to the GRCh38 reference genome with the Burrows-Wheeler Aligner (v0.7.17). Reads were grouped by unique molecular identifiers (UMIs) performed with fgbio (v0.4.0). Variant calling was performed with Platypus (v 0.8.1). Variant annotation was performed with Ensembl Variant Effect Predictor (VEP) and QIAGEN Clinical Insight (QCI). A detailed description has been previously reported (45, 46).

Prediction of pathogenicity

Individual variant pathogenicity was evaluated using SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and CADD (Combined Annotation Dependent Depletion) score (https://cadd.gs.washington.edu).

Allele frequency

For each cohort, the \textit{SAMD9} variant allele frequency was calculated by dividing the number of alleles with any specific variant by the total number of alleles sequenced. Allele frequencies were compared to data in gnomAD v2.1.1 (accessed May 2022, https://gnomad.broadinstitute.org) (47).

Analysis of \textit{SAMD9} expression

RNA expression of \textit{SAMD9} in a panel of adult human tissues was obtained from the Human Protein Atlas (https://www.proteinatlas.org) using the consensus RNA-Seq dataset raw counts (https://www.proteinatlas.org/ENSG00000205413-SAMD9/tissue) (48). Relative expression of \textit{SAMD9} in a panel of human fetal and adult tissue was adapted from the previous report of Buonocore et al., 2017 (10). Single cell RNA sequencing analysis of placental \textit{SAMD9} was evaluated using publicly available data at https://maternal-fetal-interface.cellgeni.sanger.ac.uk (49) and http://placenta.grid.wayne.edu/ (50).

Graphical representations

Graphics were generated using GraphPad Prism version 8.4.3 for Windows (GraphPad Software, San Diego, USA; www.graphpad.com). Illustrations of \textit{SAMD9} protein domains were created using Domain Graph version 2.0 (51).

Standard deviation score (SDS)

Birth SDS values, whenever not reported in the original article, were calculated using the PediTools Web calculator (https://peditools.org) (52).

Statistical analysis

GraphPad Prism version 8.4.3 for Windows (GraphPad Software, San Diego, USA; www.graphpad.com) was used to perform Chi-squared tests for \textit{SAMD9} variants affecting arginine residues, and Mann-Whitney U tests for birth weight, gestational age and birth weight SDS data. Fisher’s exact test was used to test \textit{SAMD9} variant allele frequency in the studied cohorts compared to those reported in gnomAD v2.1.1.

Results

\textbf{SAMD9} variants and endocrine features

Based on a review of the literature, a total of 116 individuals with likely pathogenic variants in \textit{SAMD9} were identified. The distribution of all variants associated either with MIRAGE syndrome or MDS is shown in Figure 1A, with secondary somatic changes in \textit{SAMD9} indicated in red. Overall, 52 (44.8%) children had features of MIRAGE syndrome (including four individuals in two families), whereas 64 (55.2%) individuals presented with MDS (including five individuals in two families) (Figure 1A). MIRAGE-associated variants tend to cluster within certain regions of the \textit{SAMD9} protein, especially the P-loop NTPase domain, whereas MDS-associated variants are distributed across the protein domain structure (Figures 1A, B). Certain “hotspot” variants and codons with recurrent changes were seen in all groups (Figure 1A). Although primary adrenal insufficiency has been considered one of the main features of MIRAGE syndrome since the identification of this multisystem disorder (41/52, 78.8%), a substantial number of children with MIRAGE features are now reported without an adrenal phenotype (11/52, 21.2%) (Figure 1C). Interestingly, changes in arginine residues are more prevalent in MIRAGE patients overall compared to MDS (21/51, 41.2%) MIRAGE; 6/65, 9.2% MDS, \(\chi^2 = 16.33, P < 0.0001\), and are potentially enriched in individuals with MIRAGE syndrome who have an adrenal phenotype, but numbers are small (18/40, 45.0% MIRAGE adrenal; 3/11, 27.3% MIRAGE non-adrenal, \(\chi^2 = 1.119, P = 0.2901\) (Figure 1D).

To address whether children without an adrenal phenotype have a less severe condition in general, we used birth weight and gestational age as surrogate “markers” of phenotype severity. Infants with MIRAGE syndrome who do not have an adrenal
phenotype tend to weigh more at birth (median weight 1515g, range 834 to 2002g) than those with adrenal features (median 1020g, range 464 to 1870g) (P < 0.05). They are delivered later (median age 34.5 weeks, range 30 to 37.3) compared to those having an adrenal phenotype (median 31 weeks, range 25 to 37 weeks) (P < 0.05) (Figures 2A–C). Birth weight SDS was not significantly different (adrenal phenotype, median -2.45, range -4.0 to -0.6; without adrenal phenotype, median -2.05; range -3.3 to -1.0).
to -0.9; P = 0.34) (Figure 2D). Taken together, these data suggest that there may be a subtle spectrum of disease phenotypes, and that adrenal features are more common in very small, preterm babies with MIRAGE syndrome.

Since the first description of MIRAGE syndrome in 2016, there has been an increasing number of publications reporting patients with MIRAGE features with variants in SAMD9, and the phenotypic spectrum of SAMD9-related conditions has now expanded. Although adrenal insufficiency was a predominant feature in early reports, the relative proportion of children without adrenal insufficiency has increased with time (Figure 3A). Whilst 46,XY boys with hypospadias are now more common than 46,XY girls with severe gonadal dysfunction, 46,XX girls remain underreported (Figures 3B, C). Several patients have been identified who have additional endocrinopathies including hypothyroidism, hypo- and hyper-glycemia, growth hormone deficiency and panhypopituitarism, although it is often unclear how extensively most children have been investigated for endocrine disorders and some may have died before these were manifest (Figure 3D).
Placental SAMD9 expression and recurrent miscarriage

Analysis of RNA expression in several adult and fetal human tissues shows high levels of SAMD9 in the esophagus, fetal adrenal, colon, bone marrow, thymus, lung and fetal testis (Figures 4A, B), which are tissues particularly affected in MIRAGE syndrome. FGR though, remains a common issue in MIRAGE syndrome and babies are often delivered due to severe FGR. SAMD9 is a growth repressor and could be directly implicated in the pathogenesis of growth restriction in the fetus itself or might affect the fetoplacental unit. To address this further, we investigated the expression of SAMD9 in human placenta using publicly available resources. Data from single cell RNA sequencing indicate that only a small population of...
annotated cells show SAMD9 expression in first trimester human placenta (Figure 5A). In a study of third trimester placenta (33-40 weeks of gestation), more extensive SAMD9 expression was seen especially in lymphoid tissues (Figure 5B). No enrichment of pathogenic nor polymorphic variants in SAMD9 was identified in DNA from early lost pregnancies (POC), or in couples who had experienced recurrent miscarriage (RM) (Table 2; Figure 6).

**SAMD9 and growth restriction**

Given the range of phenotypes associated with SAMD9 that have now been reported, we investigated the potential role of SAMD9 variants in a mixed cohort of children with FGR, SGA, and SRS (Table 1). Samples were subjected to targeted NGS and WES, with the aim of identifying any potential pathogenic variants and/or any enrichment of rare variants in SAMD9 in any of the groups. We did not find any predicted pathogenic variants in the cohorts studied, and we did not observe any significant differences in SAMD9 variant allele frequencies in our cohorts compared to those reported in gnomAD (Fisher’s exact test, all analyses $P = 1$) (Table 2 and Figure 6).

**Discussion**

MIRAGE defines a multisystem disorder caused by heterozygous missense variants in SAMD9, which is located on the long arm of chromosome 7. These variants lead to a gain-of-function of the growth repressor SAMD9, causing tissue hypoplasia and growth restriction. The natural history of the condition can be modified in the hematopoietic system at least
by revertant somatic rescue mechanisms that can occur in cis to “remove” the affected allele. These events include somatic nonsense or frameshift variants, often only present in a small proportion of sequencing reads, and progressive loss of chromosome 7 or more specifically 7q, where SAMD9 is located. Cells carrying the mutated SAMD9, which then develop the secondary somatic changes, have a clonal advantage allowing partial rescue. However, this can lead to the development of myelodysplasia in the hematopoietic system secondary to the loss of chromosome 7.

As is often the situation when new clinical associations are identified, the first MIRAGE patients reported exhibited the most severe phenotypic features. Many children died in the first few months of life or in utero. More recently additional children...
TABLE 2  SAMD9 allele frequency in the study cohorts.

| SAMD9 variants (p.) | VEP Annotation | CADD Score | Allele Frequency |
|---------------------|----------------|------------|-----------------|
|                     |                |            | gnomAD v2.1.1   | POC (n=26) | RM (n=96) | FGR (n=44) | SGA (n=20) |
| R75W                | missense       | 16.48      | 0.02            | –          | 0.01      | –          | –          |
| S86F                | missense       | <10        | 0.00            | –          | 0.01      | –          | –          |
| H143T               | missense       | <10        | 0.12            | 0.19       | 0.22      | 0.14       | 0.08       |
| F313                | synonymous     | <10        | –              | –          | 0.01      | –          | –          |
| Y320                | synonymous     | <10        | 0.38            | 0.40       | 0.53      | 0.44       | 0.45       |
| N449S               | missense       | <10        | 0.02            | 0.04       | 0.05      | 0.02       | 0.05       |
| A454T               | missense       | <10        | 0.05            | 0.04       | 0.07      | 0.07       | 0.05       |
| P466                | synonymous     | <10        | 0.00            | –          | –         | 0.02       | –          |
| T479M               | missense       | <10        | 0.06            | 0.04       | 0.07      | 0.11       | 0.05       |
| V549L               | missense       | 21.80      | 0.09            | 0.15       | 0.16      | 0.11       | –          |
| D881G               | missense       | 23.10      | 0.02            | 0.04       | 0.02      | 0.03       | –          |
| K894E               | missense       | <10        | 0.01            | –          | –         | 0.06       | 0.10       |
| N1003S              | missense       | <10        | 0.00            | –          | 0.01      | –          | –          |
| F1275               | synonymous     | <10        | 0.02            | –          | 0.02      | –          | –          |
| A1556T              | missense       | 10.12      | 0.01            | –          | 0.02      | –          | –          |

Allele frequency for the variants detected in each cohort is shown. No data are shown for the Silver-Russell Syndrome (SRS) group as no variants were detected. Allele frequency is calculated by dividing the total number of alleles carrying the variant by the total number of alleles present in each individual cohort.

ALLELE FREQUENCY

Whether adrenal insufficiency has been one of the key features of MIRAGE syndrome, with most children initially described having an adrenal phenotype. As more cases have been reported, it has emerged that an increasing number of patients do not have an adrenal phenotype, as we have shown here. In general, early adrenal dysfunction is associated with a lower birthweight and lower gestational age, as well as greater degree of gonadal dysfunction as indicated by the genital phenotype. Whether adrenal insufficiency contributes to prenatal fetal distress is unclear. Therefore, a spectrum of phenotypic severity seems to be emerging. This range of features could be linked to the underlying genetic variant; for example, on the limited data available, changes in codon 982 (p.R982C, p.R982H) seem to associate with adrenal dysfunction, whereas two children with changes in the neighboring codon (p.I983S) did not have adrenal features. More data are needed in this regard. Alternatively, some children without adrenal insufficiency could have adrenal rescue mechanisms such as somatic revertant mosaicism in progenitor cells that repopulate the gland under ACTH stimulation, but this theory is difficult to prove given the challenges in accessing adrenal tissue for investigation. On a practical level, adrenal features may be masked if a child receives steroids for another reason (e.g., chronic lung disease or infection), although to our knowledge this was not the situation in the children described here. Finally, whether any of these individuals develop adrenal insufficiency with time is unknown, so clinical vigilance and potentially periodic screening of adrenal function may be warranted.

The association between growth restriction and primary adrenal insufficiency is not limited to MIRAGE syndrome. Similar growth restriction syndromes characterized by adrenal insufficiency, FGR and other features, include IMAGe syndrome (37). Here we show that children with MIRAGE features and growth restriction are more likely to have recurrent or “hotspot” variants compared to children with MDS, and more likely affecting amino acids in the P-loop NTPase domain or arginine residues, suggesting a potential emerging genotype-phenotype correlation. Of note, a SAMD9/9L effector domain (codons 134-385, which include the Alba domain) in association with DNA has recently been crystalized (53), but this structure does not contain most of the key hotspot codons relevant to MIRAGE syndrome.

Adrenal hypoplasia has been one of the key features of MIRAGE syndrome, with most children initially described having an adrenal phenotype. As more cases have been reported, it has emerged that an increasing number of patients do not have an adrenal phenotype, as we have shown here. In general, early adrenal dysfunction is associated with a lower birthweight and lower gestational age, as well as greater degree of gonadal dysfunction as indicated by the genital phenotype. Whether adrenal insufficiency contributes to prenatal fetal distress is unclear. Therefore, a spectrum of phenotypic severity seems to be emerging. This range of features could be linked to the underlying genetic variant; for example, on the limited data available, changes in codon 982 (p.R982C, p.R982H) seem to associate with adrenal dysfunction, whereas two children with changes in the neighboring codon (p.I983S) did not have adrenal features. More data are needed in this regard. Alternatively, some children without adrenal insufficiency could have adrenal rescue mechanisms such as somatic revertant mosaicism in progenitor cells that repopulate the gland under ACTH stimulation, but this theory is difficult to prove given the challenges in accessing adrenal tissue for investigation. On a practical level, adrenal features may be masked if a child receives steroids for another reason (e.g., chronic lung disease or infection), although to our knowledge this was not the situation in the children described here. Finally, whether any of these individuals develop adrenal insufficiency with time is unknown, so clinical vigilance and potentially periodic screening of adrenal function may be warranted.
unknown etiology without prematurity or growth restriction (57).

In addition to the well-described gonadal defects that can affect both the testis and ovary (15), a range of additional endocrine features have been reported with MIRAGE syndrome and SAMD9 disruption. These include glucose dysregulation, hypothyroidism, and hypothalamo-pituitary issues such as growth hormone insufficiency and panhypopituitarism. Sometimes these features may be influenced by the clinical status of the child (especially in pre-term babies) or by hypocortisolism. However, it does seem that additional endocrine features can occur, and these may well be underreported if they are not regularly assessed or if children do not survive long enough for them to manifest. More long-term data are needed in this regard, including whether puberty is affected in MIRAGE syndrome, and how best to optimize growth potential throughout childhood and adolescence.

As prenatal and postnatal growth issues are common in MIRAGE patients, and infants are often delivered early due to severe FGR, we investigated a possible link between SAMD9 and pregnancy loss. Data from single cell RNA sequencing show low levels of SAMD9 expression in first trimester human placenta, suggesting that it is unlikely to be a driver of growth restriction via a placental mechanism during that time, but rather via a fetal specific system. However, higher expression was seen later in pregnancy, and histological evaluation of placenta tissues from SAMD9 patients showed poorly developed distal villous trees with widening of the intervillous space, suggesting abnormal development (22). These findings could indicate a role of SAMD9 in both the placenta and fetus, having therefore a dual function in prenatal growth disruption. In our analysis of a limited number of available samples, we did not identify likely pathogenic SAMD9 variants in early POC tissue (spontaneous pregnancy loss), nor in genomic DNA of couples who had early recurrent miscarriages (RM). Mosaic placental mechanisms occurring de novo in the fetus could not be excluded. This contrasts with CDKNIC, which shows marked expression in first trimester placental tissue, highlighting a potential role for fetal growth and survival (45). Taken together, the higher placental expression of SAMD9 in later pregnancy coupled with evidence of abnormal placental development in affected patients, could be contributing factors to poor growth and fetal distress seen in
MIRAGE syndrome, and a reason these babies are delivered early.

SAMD9 variants can be found in children born SGA (22) and interestingly, they can be inherited from asymptomatic parents, exhibiting different rescue mechanisms from offspring (20). This led us to analyze SAMD9 in samples from a cohort of children with FGR, SGA and SRS, to investigate whether potential predicted pathogenic variants could be found that could explain the growth restriction phenotype. We did not find any primary or secondary somatic variants, nor did we observe an enrichment of more common variants in SAMD9 in the cohort studied, suggesting that SAMD9 is not a frequent driver of growth disorders unless other features are present.

This study has several important implications. Establishing the range of endocrinopathies in MIRAGE syndrome is essential to reach a quick diagnosis and tailor patient treatment, as adrenal insufficiency is a life-threatening condition and thyroid hormone imbalance can affect brain development and growth. It is also important to define and treat other features, such as dry eyes, feeding difficulties and esophageal reflux, periodic fever, immune dysfunction and gastrointestinal effects, amongst other considerations (11). Establishing a diagnosis of a SAMD9-related condition enables monitoring of monosomy 7, as a secondary somatic event to remove the primary driver, and consequent potential MDS risk. Finally, the features of MIRAGE syndrome can be very non-specific and often seen in sick babies in neonatal medicine delivered prematurely with growth restriction. Raising the awareness of this condition and having greater access to rapid genetic sequencing could be essential to identify children who harbor pathogenic SAMD9 variants, and to identify some of the “missing” cohort of 46,XX girls with this condition. Indeed, more widespread use of whole exome/ genome sequencing for children with growth restriction and associated features is likely to identify more children with MIRAGE-associated conditions and provide more information on the range of phenotypic features.

This study has several limitations: 1) Following the literature review, several case reports lacked complete information such as BW and GA, especially for patients carrying SAMD9 variants in the MDS cohort. Furthermore, detailed genetic studies of genotype-phenotype co-segregation were not always described in MDS patients, and functional work is limited suggesting that some false positives may have been reported. 2) As discussed above, endocrine features may also be overlooked and not reported, or they may develop later with time. There is therefore a need for a life-course data analysis to be able to capture the full range of phenotypes associated with SAMD9 variants. 3) Due to a relatively small cohort of miscarriage tissues and RM couples, we might have missed de novo variants, but potentially parental germline mosaicism would be enriched, and this is important as there are several sibling pairs with SAMD9 variants reported. 4) The FGR cohort analyzed in this study was relatively small. Analysis therefore does not exclude SAMD9 as a potential pathogenic gene in this cohort, but suggests it is not a common occurrence.

SAMD9-associated variants comprise a wider spectrum of phenotypes than originally reported, including children with neonatal growth restriction and multisystem features but without adrenal insufficiency. Endocrinopathies are also emerging, though these are possibly overlooked. Indeed, as these patients may present to a diverse range of health care professionals, and with features beyond those in the classic MIRAGE acronym, perhaps referring to “SAMD9-related syndromes” in the future may be more appropriate. We have also shown that SAMD9 variants are not common in fetal growth disorders unless additional features are present. Nevertheless, monitoring severe FGR children remains essential and has important implications in reaching a specific diagnosis for personalized multidisciplinary management.

Data availability statement

The original contributions presented in the study are publicly available and can be found here http://doi.org/10.17605/OSF.IO/WMVXY (39). These data include an overview of SAMD9 publications and linked reported variants, and a summary of all SAMD9 variants identified using the HaloPlex, Nonacus and WES platforms.

Ethics statement

The studies involving human participants were reviewed and approved by BBB Research Ethics Committee references: 09/H0405/30 and 09/H0405/30+5; Moore cohort reference: 2001/6029. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

Author contributions

JS and FB designed the targeted NGS panels. JS carried out NGS experiments. MI and ID conducted bioinformatic pipeline analysis. SS provided variants for the SGA samples. NS coordinated samples and clinical data from the BBB. EW contributed to the clinical evaluation of the article. GM is the founder of the BBB and Moore Cohort and provided all the samples and conceptual input. JA and FB undertook literature reviews, conceptualized, and wrote the article. FB conducted the molecular analysis, produced figures and tables. All authors contributed to manuscript revision, read, and approved the submitted version.
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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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