Original Article

Phenotype from *SAMD9* mutation at 7p21.1 appears attenuated by novel compound heterozygous variants at *RUNX2* and *SALL1*

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Abstract: Sterile alpha motif domain-containing protein 9 (SAMD9) is a regulatory protein centrally involved in cell proliferation and apoptosis. Mapped to 7p21.1, variants in *SAMD9* have been reported in <50 pediatric cases worldwide, typically with early lethality. Germline gain-of-function *SAMD9* variants are associated with MIRAGE Syndrome (myelodysplasia, infection, restricted growth, adrenal hypoplasia, genital anomalies, and enteropathy). Spalt like transcription factor 1 (SALL1) is a zinc finger transcriptional repressor located at 16q12.1 where only two transcript variants in *SALL1* are known. *RUNX2* (6p21.1) encodes a nuclear protein with a Runt DNA-binding domain critical for osteoblastic differentiation, skeletal morphogenesis, and serves as a scaffold for nucleic acids and regulatory factors involved in skeletal gene expression. RUNX2 and SALL1 are thus both 'master regulators' of tissue organization and embryo development. Here, we describe exome sequencing and copy number variants in two previously unknown mutations—R824Q in SAMD9, and Q253H in SALL1. A new multiexon 3' terminal duplication in *RUNX2* is also reported. This is the first known phenotype characterization for the intersection of all three variants in a healthy 46,XX adult. Focusing on developmental progress, ultrastructural renal anatomy, and selected reproductive aspects, we describe this unique genotype diagnosed incidentally during Covid-19 illness. Individual disruption in *SAMD9*, *RUNX2*, or *SALL1* would be expected to give a bleak prognosis. However, the convergence discovered here appears to dampen severe pathology, perhaps by cross-gene silencing of effects normally deleterious when such changes occur alone.

Keywords: SAMD9; RUNX2; SALL1; ovarian anatomy; renal structure

1. Introduction

Mutations in SAMD9 (sterile alpha motif domain-containing protein 9) are usually recognized early in life, as adaptive immune response impairment leads to chronic childhood infections and high mortality by age 10 [1,2]. *SAMD9* maps to chromosome 7q21.2, a region frequently deleted in myeloid malignancies. Narumi *et al* [3] were the first to implicate germline missense *SAMD9* mutations as causative for a rare condition known as MIRAGE syndrome (*i.e.*, myelodysplasia, infection, growth restriction, adrenal hypoplasia, genital anomaly, and enteropathy). Such mutations were later identified in ~18% of inherited bone marrow failure and myelodysplasia cases [4]. Regarding *RUNX2*, about 200 different mutations are known and most are seen with cleidocranial dysplasia, an autosomal dominant skeletal disorder featuring clavicular dysmorphia, increased head circumference, large fontanels, dental anomalies, short stature, and sometimes hand malformations [5]. Spalt like transcription factor 1 gene (*SALL1*) modulates onset and progression of human tumors, with variants now known to be associated with Townes-Brocks syndrome (*i.e.*, anal, renal, limb, and ear anomalies) [6,7]. While disruptions in *SAMD9*, *RUNX2*, and *SALL1* have each been separately described, this report is the first to present data on simultaneous variants in all three.

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2. Clinical Presentation

This 18yr-old nonsmoking Caucasian female sought a second opinion consult regarding irregular menses. Academic progress was normal through high-school, with extracurricular activities including team sports and music. Accompanied by her mother, the patient appeared developmentally normal. Review of the prenatal chart was notable for small for gestational age diagnosis in the third trimester. A genetic amniocentesis was normal. She was delivered by Cesarean at 34 weeks' gestation with birth weight 1700g. Placenta and umbilical cord were grossly normal. Bilateral choanal atresia was diagnosed while in neonatal nursery, and was successfully repaired by age three months.

Recent medical history was unremarkable, although mild intermittent macrocytic anemia was occasionally noted on CBC and required no treatment. There was no history of electrolyte imbalance. Blood type was O+. Menarche was at age 11, and normal ovarian, uterine and cervical anatomy was noted on a prior pelvic CT. Six months before assessment, the patient was evaluated elsewhere for persistent headache, low grade fever, diarrhea, emesis and fatigue. Pregnancy test was negative. Covid-19 screen was initially negative, but oral vancomycin was started for *C. difficile* which was found incidentally. Symptoms failed to resolve, and temperature increased to 39.7°C within 2d when repeat Covid-19 testing was positive. By now underweight, the patient was admitted to hospital where supportive care was provided in an isolation unit; oral vancomycin was adjusted to i.v. administration. Three days later, her BMI was 16.8 and fever, diarrhea and vomiting persisted. Proteinuria was noted with high serum creatinine (3.5mg/dL). The diagnosis was revised to Covid-19 associated multisystem inflammatory syndrome in children (MIS-C) and the patient was transferred to ICU. Next, vancomycin was discontinued and remdesivir, azithromycin, dexamethasone, and intravenous immunoglobulin therapy began [8]. While in ICU, the patient's ESR, C-reactive protein, and D-dimer level (all of which had been markedly elevated) began to decline. After a 15d hospitalization, she was discharged home with a recommendation for outpatient renal biopsy. This was completed along with a comprehensive genetics panel. Her gastrointestinal symptoms resolved as activities returned to normal, yet appetite remained low (thought to be Covid-19 sequela).

Renal biopsy showed focal global and partial glomerulosclerosis, extensive foot process effacement, as well as mild interstitial chronic inflammation (see Figure 1). These changes were regarded as consistent with recent Covid-19 infection. Exome sequencing identified three previously unpublished variants (see Figure 2). Protein variation effect analysis [9] was performed to estimate impact of amino acid substitution on resultant protein bioactivity by delta alignment score [10]. While the new *SAMD9* variant was associated with a neutral effect (-0.5), at *SALL1* the variant was considered deleterious (-4.08). Data were insufficient to develop an estimate for the large *RUNX2* duplication (see Table 1). Analysis of mtDNA was normal.

	coding DNA	protein product	coordinates	var.effect [a]	parental data
SAMD9	2471 G>A	Arg824Gln R824Q	7:92732940	-0.5	*de novo*
RUNX2 [b]	[*dup*]				mat.dup.
SALL1	759 A>T	Gln253His Q253H	16:51175374	-4.08	mat.var.

Notes: Variants reported are inherited as heterozygous/autosomal dominant. [*dup*] = gene duplication. [a] Calculated protein variation effect analysis (PROVEAN) [b] 3' end duplication corresponds to arr[GRCh37] 6p21.1 (45459496_45515207)x3; same *RUNX2* variant also identified in mother.

Table 1. Summary of variant, protein, and genomic data present in 46,XX non-syndromic SAMD9.

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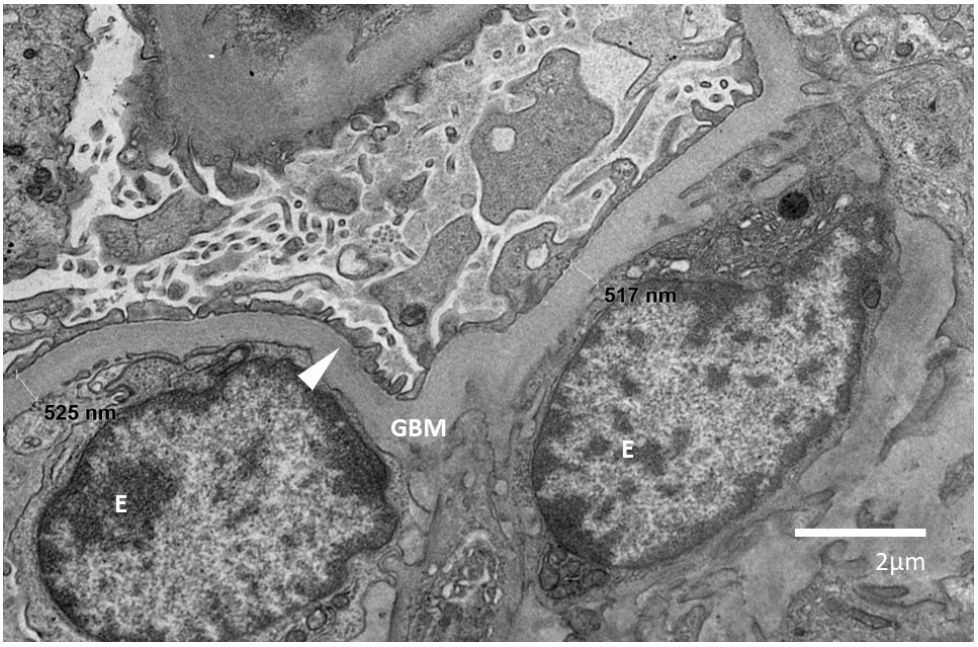

Figure 1. Transmission EM (1500x) view of renal tissue in a healthy 46,XX patient with variants at SAMD9, RUNX2, and SALL1. Moderate focal global glomerulosclerosis is noted with segmental, irregular podocyte foot process effacement (arrow). Minimal basement membrane thickening is also present with no significant endothelial edema. Immunofluorescence stains were negative for IgA, IgM, C1q, C3, kappa, lambda, fibrinogen or properdin. E=endothelial cell, GBM=glomerular basement membrane. *Photo courtesy*: Winston Hooker, MHA; LSU Health Science Center (Shreveport LA)

3. Testing protocol

Genomic DNA analysis was performed on cells sampled from proband and both parents by buccal swab technique [11]. Exomes and flanking splice junctions were captured using the IDT xGen Exome Research Panel v1.0 (Integrated DNA Technologies, Coralville, Iowa USA). Massively parallel NextGen sequencing was done on an Illumina platform with ≥100bp paired-end reads. These were aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis tool [12]. Additional sequencing and variant interpretation were applied as described previously [13]. Variant classification criteria are available at GeneDx® ClinVar page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

4. Discussion

Occurrence of *SAMD9, RUNX2* and *SALL1* mutation in humans is exceedingly rare and information on these events mainly comes from small series or case reports. Such data provide knowledge of gene function, particularly when variants are linked to specific phenotypes. Since SAMD9 is a key regulatory protein crucial for normal development, it is unsurprising that variants in the responsible gene would result in an 'orphan disease' condition in children with a dismal prognosis [14]. Yet the full significance of any discovered variant is best appreciated by consideration of the entire genome. The convergence we report here is believed to be the first instance where unique variants in *SAMD9, RUNX2* and *SALL1* all appear together. The phenotype assessment adds to the understanding of these loci, and suggests a possible integrative effect on overall function.

For our patient, the provisional diagnosis of MIRAGE syndrome seemed a poor fit given her general clinical picture and developmental history. While the association

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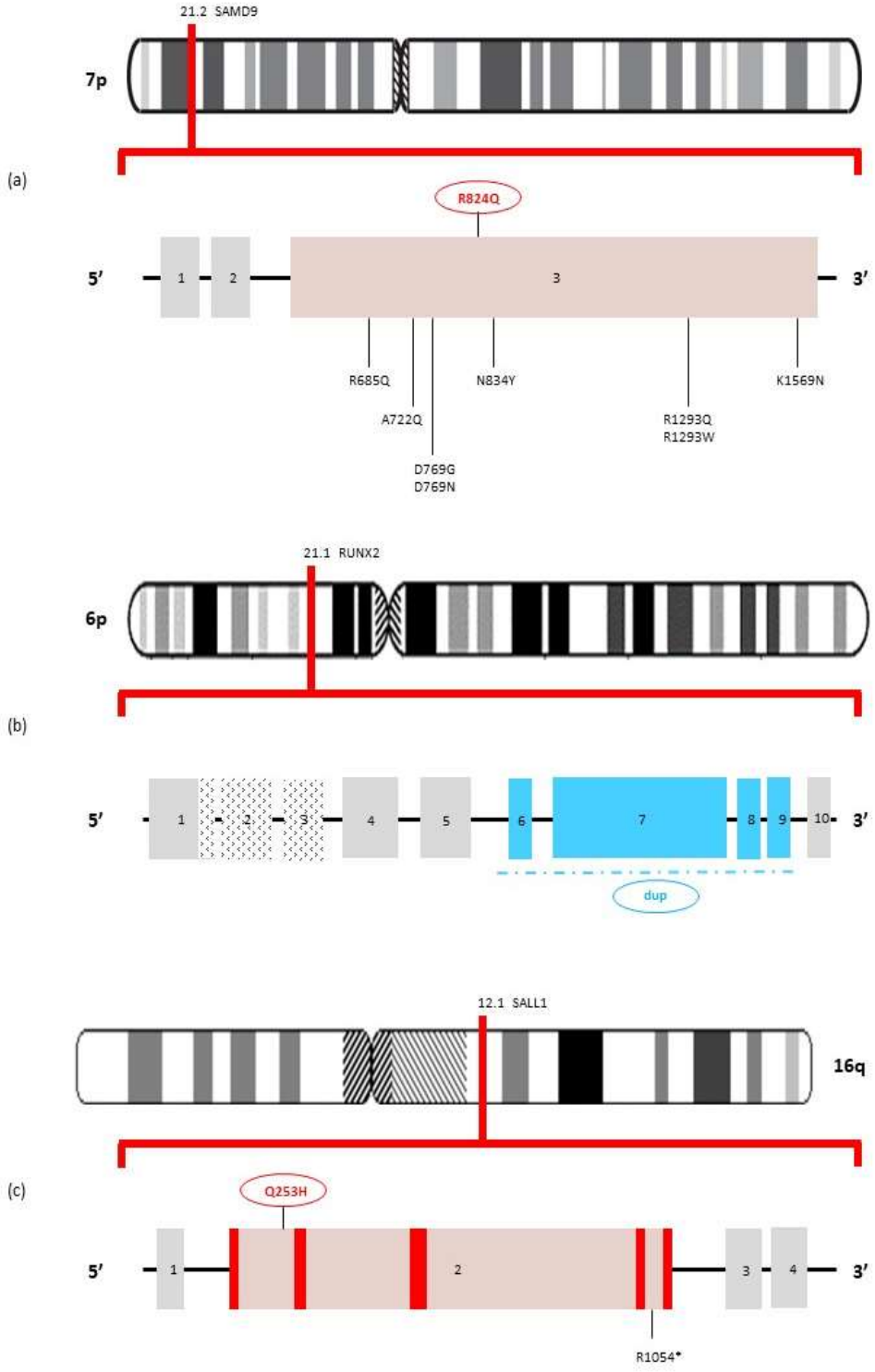

Figure 2. Schema for selected representative mutations and (a) new variant at 7p21.1 *SAMD9* (R824Q, red oval) NM_017654.3. Also shown are (b) new multiexon *RUNX2* duplication (blue oval) at 6p21.1 (NM_001024630.3) and (c) new variant at 16q12.1 *SALL1* (Q253H, red oval) NM_002968.2. The *RUNX2* and *SALL1* variants were also found in proband's healthy mother.

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between *SAMD9* and MIRAGE syndrome is established [3], in our case a theory was required to account for multiple missing syndromic elements. Prior MIRAGE syndrome research emphasized germline mutations, which was not proven here. Other possibilities included a reversion mutation [15], or perhaps our variant in *SAMD9* was differentially operant on growth networks in multiple cell contexts. This would align with how alterations in the Ras/mitogen-activated protein kinase (Ras/MAPK) pathway are considered to result in a spectrum of pathologies with distinct clinical phenotypes [16,17]. Cross-regulation by variants elsewhere could not be dismissed, however. Accordingly, attention was next turned to *RUNX2* and *SALL1*.

Spalt-like transcription factors (SALLs) are highly conserved proteins which govern embryo development, apoptosis, angiogenesis, invasion, and metastasis [18]. SALL1 is a transcription factor which mediates organogenesis and cell differentiation [19]. Since gene expression is slowed when DNA is loosely packed, and SALL1 can alter this by influencing how tightly DNA is packaged, the presence of this new *SALL1* variant gained additional relevance. Moreover, an instance where *SALL1* influences gene operation is already known from its role as a tumor suppressor in breast cancer, which is down-regulated in estrogen receptor, progesterone receptor, and epidermal growth factor receptor-2 'triple negative' breast cancer patients [20].

Runt-related transcription factor 2 (RUNX2) regulates osteogenesis and shares common signaling pathways in other tissue development. It is known to co-occur in several epithelial and mesenchymal cancers, linked by multiple cancer-related proteins and microRNAs [21]. Of note, experts in China recently found a role for *SALL1* in differentiation of murine odontoblast lineages, specifically involving *RUNX2* [22]. Our data support a coordinated regulatory role involving both *RUNX2* and *SALL1*, offering further justification for why these genes should be considered together.

Several issues remain open and thus limit this report. For example, although examination of renal ultrastructure showed nonspecific inflammatory changes, this may have been related to Covid-19 rather than any gene variant identified here (*i.e.*, comparison vs. pre-infection biopsy sample was not possible). Also, images confirming grossly normal pelvic anatomy cannot forecast reproductive capacity into adulthood. And since our evaluation was confined to exon sequencing, any non-coding DNA mutation would be missed by this technique [23,24]. Yet, the identification of these unexpected variants in *SAMD9*, *RUNX2* and *SALL1* in a clinical setting of normal development offers new insights into how these loci may operate in concert.

In summary, the pathogenic effects of these *de novo* variants in *SAMD9*, *RUNX2* and *SALL1* are expressed differently together than expected if any were present separately. Careful longitudinal follow-up is warranted and data from further assessments will be reported when available.

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