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ORIGINAL ARTICLE

TREX-1-Related Disease Associated with the Presence of Cryofibrinogenemia

C. Paradis[1] · M. Cadieux-Dion[1,2] · C. Meloche[1] · M. Gravel[1] · J. Paradis[3] · A. Des Roches[4] · G. Leclerc[5] · P. Cossette[1,3] · P. Begin[1,3,4,5]

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Abstract

Purpose Cryofibrinogenemia is a rare cryopathy presenting as acrocyanosis following exposure to cold. Familial presentation has been described but the underlying molecular cause remained undetermined.

Methods Forty (40) members from a large family with an initial diagnosis of familial cryofibrinogenemia were interviewed and examined to determine affected status and collect DNA. Exome sequencing was performed on three affected individuals from distinct branches of the pedigree.

Results Seventeen (17) family members reported a history of acrocyanosis with cold exposure. None reported symptoms were suggestive of lupus. Exome sequencing of three subjects identified the heterozygous mutation D18N in the *TREX1* gene which was then confirmed by Sanger sequencing in all affected as well as 2 unaffected family members. The mutation is already being associated with familial chilblain lupus erythematosus (CHLE), and a systematic review of literature was undertaken to compare reports of familial CHLE and cryofibrinogenemia. Both entities were found to share highly similar clinical presentations suggesting they are part of a same syndrome in which cryofibrinogenemia and lupus manifestations have variable penetrance.

Conclusions Familial cryofibrinogenemia without lupus should be added to the spectrum of *TREX1*-related disease.

Keywords Cryofibrinogenemia · Cryofibrinogen · TREX1 · Chilblain · Lupus · Genetics · Familial · Acrocyanosis · Auto-inflammatory · Auto-inflammation

Abbreviations

AGS	Aicardi-Goutières syndrome
CF	Cryofibrinogenemia
CHLE	Chilblain lupus erythematosus
COL7A	Collagen 7 alpha
IRF3	Interferon regulatory factor 3
LE	Lupus erythematosus
MUC6	Mucin 6
SAVI	STING-associated vasculopathy with onset in infancy
ssDNA	Single-stranded DNA
TREX1	Three-prime repair exonuclease 1
USP19	Ubiquitin-specific peptidase 19

C. Paradis and M. Cadieux-Dion are co-first authors

✉ P. Begin
philippe.begin@umontreal.ca

[1] CHUM Research Center, Université de Montréal, Montreal, Canada

[2] Center for Pediatric Genomic Medicine, Children's Mercy Hospital, Kansas City, MO, USA

[3] Department of Medicine, Centre Hospitalier de l'Université de Montréal, Montreal, Canada

[4] Department of Pediatrics, Centre Hospitalier Universitaire Ste-Justine, Montréal, Canada

[5] Department of Medicine, Chicoutimi Hospital, Saguenay, Canada

Introduction

Cryofibrinogenemia (CF) is a rare cryopathy defined by the presence of a cold-induced precipitate found in plasma which, unlike with cryoglobulinemia, is absent from serum [1]. The CF precipitate becomes soluble again when brought back at body temperature. When symptomatic, CF mainly presents as skin lesions in cold-exposed areas (typically buttocks, hands, feet, ears, and nose), ranging from purpura, livedo reticularis or Raynaud's phenomenon to ulceration, gangrene, and ischemic necrosis in more severe cases. Diagnosis is confirmed by the detection of cryofibrinogen in plasma. This is not

performed routinely as it is technically challenging. Collection tubes need to be pre-heated and kept at body temperature from blood draw to plasma isolation to prevent CF from precipitating in the collection tube and being lost in the blood cell pellet of the initial centrifugation before it can be detected.

Most symptomatic cases of CF are secondary to either acute infection, collagen disease and vasculitis, malignancy, or thromboembolic disorder, with auto-immunity accounting for two-thirds of secondary cases [2]. Subclinical levels of CF can present asymptomatically in up to 3% of hospital population, although these patients are 4 to 5 times more likely to have invasive malignancy, severe thromboembolic event, or to die during their stay [1, 3]. Primary cases (essential CF) are less frequent and symptoms are more likely to limit to the skin with a lower risk of venous or arterial thrombosis [4]. In a cohort of essential CF followed for 5 years, 27% of patients were later diagnosed with lymphoma, suggesting many primary cases may in fact be secondary to an undetected chronic disorder [5]. Primary cases of CF are mostly sporadic although there are reports of familial primary CF, the underlying genetics of which having yet to be defined [1, 6, 7].

Chilblain lupus erythematosus (CHLE) is another disease defined by acrocyanosis but unlike primary CF it associates with lupus manifestations and the absence of cryoprecipitate. Diagnosis of sporadic CHLE requires two major criteria ((1) cold-induced lesions and (2) skin biopsies compatible with lupus erythematous (LE)) and at least one minor criteria ((1) systemic LE, (2) discoid LE, (3) response to anti-LE therapy, or (4) absence of cryoprecipitate), although it is generally accepted that the absence of cryoglobulin, cold agglutinin, or cryofibrinogen is required for diagnosis [2]. Familial cases of CHLE have been shown to be associated with heterozygous mutations in the *TREX1* gene.

Here, we describe a large familial cohort initially diagnosed with familial CF in which exome sequencing identified a heterozygous mutation in the *TREX1* gene as the underlying cause. As none of the family members had clinical or pathological manifestations of lupus, this finding argues for a revision of the clinical definition of *TREX1*-related disease.

Index Case

The index case was a 42-year-old French-Canadian male who presented with lesions on his extremities, knees, and hips that appeared every fall and disappeared during the spring. Physical examination showed scar tissue on both hips, purple-blue livedoid discolorations with crusts on both knees, and purple-blue macules with occasional crusts on finger and toe tips. Two brothers had similar symptoms. None had shown other clinical manifestations of lupus erythematous, discoid lupus, or another inflammatory condition. Complete blood count, coagulation, protein electrophoresis, and sedimentation rate were normal. Biochemistry, including creatinine, C protein, S protein, cholesterol, and hepatic enzymes, were also within normal limits. Serology was negative for hepatitis B and C, mononucleosis, VDRL, rheumatoid factor, C-reactive protein, anti-nuclear, anti-DNA, and antiphospholipid antibodies. While no cryoprecipitate was identified in serum, cryofibrinogen was identified in the patient's plasma. Skin biopsies of lesions showed thickened capillary walls with lymphocytic-histiocytic perivascular infiltrate without basal membrane thickening or vacuolization. Immunofluorescence was negative except for the presence of fibrinogen in and around capillaries.

One month later, a 42-year-old female without any apparent link to the first family consulted in a neighboring city with similar complaints of acrocyanosis and ulcers on the nose, ears, and knees [3]. Her two children suffered from the same symptoms, and she could trace her ancestors with the disease. The work-up showed similar results to the first case. Ascending genealogy revealed that this nuclear family was related to the previous one with common ancestors originating from the Magdalen's islands, Quebec, Canada.

We hypothesized that an exome sequencing approach focusing on individuals from distant branches of this large pedigree would reveal the underlying genetic cause of what was at the time defined as primary familial CF.

Methods

Study Participants and Samples

Family members of the second index case were interviewed to determine affected status and genealogy. Older stems of the genealogical tree were determined by consulting the BALSAC database which includes family records covering the province of Quebec since the seventeenth century. Forty (40) family members over 3 generations were interviewed and examined to determine affected status and their DNA was collected. All collected cases underwent dermatological examination. After participants provided written informed consent, genomic DNA was extracted from blood samples using standard protocols. This project was approved by the research ethics committee of the Centre Hospitalier de l'Université de Montreal. The participants received no financial compensation.

Cryofibrinogen Measurement

Whole blood was collected for cryoprecipitate analysis from the index case and two other individuals (IV-19, IV-30, V-15, Fig. 1). The blood was sampled into 2 plain glass tubes at 37 °C; one tube contained 3.8% sodium citrate solution, the other tube contained no anti-coagulant. The tubes were

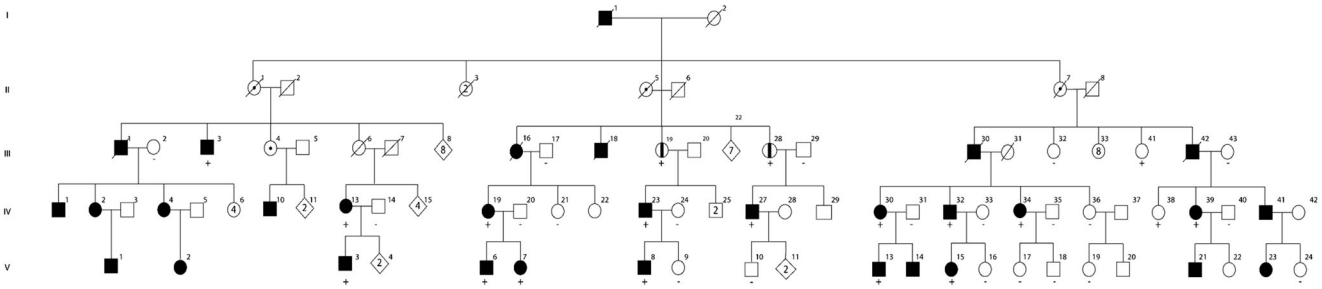

Figure 1 Pedigree of a French-Canadian family presenting with cryofibrinogenemia secondary to TREX1 deficiency. The mutation p.D18N in the gene TREX1is found in 19 individuals. – no mutation; + mutation; / deceased; black fill, affected; white fill, unaffected; black dot, carrier; black stripe, possibly affected

incubated at 37 °C for 1 h and then were centrifuged at 1500*g* for 30 min to separate plasma and serum from cells. Plasma and serum samples were kept at 4 °C for up to 72 h. Tubes were again centrifuged at 1500*g* for 30 min and examined for presence of cryoprecipitate as previously described [3]. The cryoprecipitate from one sample (V-15) was suspended in distilled water and protein content was dosed by spectrophotometry.

Candidate Gene Sequencing

We first screened the complete open reading frame of FGA (NM_000508), FGB (NM_005141), and FGG (NM_000509 and NM_021870) (primers available upon request) in one affected individual from the family (V-15). We then amplified all the coding regions of these genes by polymerase chain reaction (PCR). The primers were designed approximately 50 base pairs from the exon/intron junctions to analyze the whole sequences, including splice sites. PCR primer pairs were designed using the software Exon primer from the UCSC Genome Browser database. The amplicons were run on the ABI3730 automatic sequencer and studied by using Mutation Surveyor.

Exome Sequencing and Validation of Variants

Exome sequencing was performed on three affected individuals from distinct branches of the pedigree (V-6, V-15, IV-39, Fig. 1). Genomic DNA (5 μg) was fragmented and enriched (SureSelect Human AllExon V4 kit; AgilentTechnologies). The prepared library was sequenced with paired-end reads (HiSeq 2000; Illumina). The reads were mapped against the human genome (hg19) (Burrows-Wheeler Aligner). Reads with unique mapping location were kept for downstream analysis. Genome Analysis Tool Kit 13 and ANNOVAR 14 were used for the calling of single-nucleotide variations, small insertions, and small deletions (indels). Detected variants were filtered against dbSNP132 as well as individuals from our in-house exome database. For the validation of next-generation sequencing variants, primers were designed with the software Primer 3 to amplify the coding region encompassing the putative variation.

Systematic Literature Review

A systematic review of published literature was conducted with the aim of identifying previous reports of familial occurrence of either CHLE or primary familial CF. A computerized search was performed to identify all relevant publications from PubMed database. The following keywords or terms were used: primary cryofibrinogenemia, *TREX1*-related disease, and chilblain lupus. Full-length papers and case reports were considered for inclusion. The database was last searched on September 26, 2018. In addition to the computerized search, we manually reviewed the bibliography of all included articles to ensure complete inclusion of all relevant studies.

Results

Study Participants

Common ancestry of both index cases was confirmed by the BALSAC database and complete familial pedigree of the second family was mapped out (Fig. 1). Familial interviews allowed retracing of 34 individuals reporting a history of cold-induced acral lesions (Fig. 2). Of these, 17 participated to the interviews and provided genetic samples. In addition, 21 unaffected family members also provided DNA samples. Clinical phenotype of the 17 symptomatic participants is summarized in Table 1. There was no history of symptoms compatible with lupus erythematosus, although 6 of them reported a history of mechanical knee pain. None of the participants reported history of kidney disease or of neurological involvement (learning

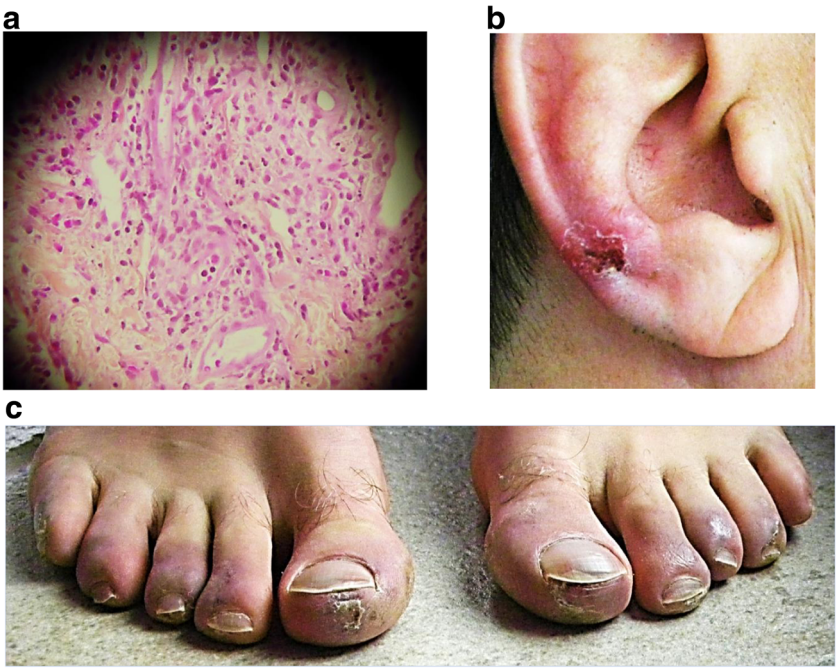

Fig. 2 Skin lesions from family member with the p.D18N mutation in TREX1. Skin biopsy showing perivascular lymphocytic infiltrate (**a**). Typical cold-induced lesions on the ear and toes (**b**, **c**)

disability, spasticity, dystonia), although specific brain imaging was not performed. Transmission of the disease phenotype was compatible with an autosomal dominant transmission pattern.

Cryofibrinogen Measurement

Laboratory evaluation showed a positive plasma cryofibrinogen in the three individuals which were tested.

Table 1 Clinical manifestations of 19 individuals carrying the p.D18N mutation in the gene TREX1. (+) positive; (−) negative; (±) possible; n/a, not available

ID	Sex	Age	Lesions	Mutation status	Age of onset	Localization of lesions
Mutation carriers with history compatible with disease						
III.28	F	75	±	+	n/a	n/a
V7	F	n/a	+	+	n/a	n/a
IV.39	F	56	+	+	Childhood	Finger, toes, ears
IV.30	F	55	+	+	Childhood	Toes, feet
IV.34	F	53	+	+	12 years	Feet, hand, ears
III.3	M	79	+	+	6 years	Ears
IV.19	F	55	+	+	7 years	Nose, ears, feet, fingers, hips, knees, face
V.6	M	27	+	+	1 years	Hands, ears, feet
III.19	F	74	±	+	n/a	n/a
V.13	M	24	+	+	2 years	Hands, feet
IV.32	M	52	+	+	15 years	Ears, feet, hands
V.15	F	19	+	+	6 years	Fingers, toes
IV.13	F	43	+	+	12 years	Thigh
V.3	M	15	+	+	3 years	Feet, ears, cheek
V.8	M	15	+	+	n/a	n/a
IV.23	M	45	+	+	n/a	n/a
IV.27	M	41	+	+	n/a	n/a
Mutation carriers without history compatible with disease						
IV.38	F	59	–	+	–	–
III.41	F	68	–	+	–	–

Plasma concentration of cryofibrinogen was measured at 850 mg/L in subject V-15.

Genetic

No mutation was identified after sequencing the complete open reading frame of FGA, FGB, and FGG in subject V-15.

Exome sequencing of V-15, V-6, and IV-39 revealed four shared, low-frequency coding genetic variations. Among those, we identified one missense variant (chr11: 1017744 T > C, NM_005961, c.A5057G, p.N1686S) and one synonymous variant (chr3: 49154642 C > T, NM_001199162, c.G729A, p.E243E) in the genes MUC6 and USP19, respectively. MUC6 encodes for a member of the mucin family and is involved in the epithelial cytoprotection in the gastrointestinal tract [7], whereas USP19 encodes for a deubiquitinating enzyme widely expressed in the body [8]. Neither of those mutations were considered as being linked to the clinical presentation.

A transition was also identified in COL7A1 (chr3:48620475 G > A, NM_000094, c.C4489T, p.R1497C). Finally, we found a missense mutation (p.D18N) in the *TREX1* gene located on chromosome 3 (chr3: 48508106 G > A, NM_033629, c.G52A, p.D18N) (Fig. 2). The *TREX1* and the COL7A1 variants were sought by Sanger sequencing in the rest of the pedigree, and were found among 16 additional individuals (12 affected, two possibly affected, and two asymptomatic individuals).

Systematic Literature Review

The database search identified 460 articles. Out of those, there were 10 reports of families diagnosed with chilblain lupus and 3 reports of families with primary CF [8–16]. The family characteristics are summarized in Table 2. All families were reported to have similar dermatological and pathological findings. Seven (7) out of the 10 families with CHLE had markers suggestive of a rheumatological process. Three primary cases of CF have been documented with familial presentation [6, 7, 17].

Van Geest et al. reported a mother and 2 of her 3 children presenting with acrocyanosis on their nose and cheeks after cold exposure (resolving after becoming warm). All subjects tested positive for cryofibrinogen without any other manifestations of auto-immunity [6].

Wulffraat et al. reported three distinct families with essential CF with children as index cases. Two presented with the classical cold-induced acrocyanosis. One also presented arthralgia in his hands and feet, generalized muscle weakness, relapsing fever, and vasculitis on pathology. All subjects were tested positive for cryofibrinogen and negative for antinuclear antibodies. Many family members also reported acrocyanosis. Interestingly, 2 symptomatic parents were tested negative for cryofibrinogen, suggesting it may only be an epiphenomenon of another cold-related disorder.

Lolin et al. reported a patient who had CF manifest as nephrotic syndrome after anesthesia. All family members were positive for CF and developed acrocyanosis when exposed to low temperatures for the first time [17]. None of the three reports investigated the underlying genetic cause of familial CF.

Discussion

The *TREX1* gene (three-prime repair exonuclease 1) encodes for a major non-processive 3′- > 5′ DNA exonuclease that digests single-stranded (ssDNA) and double-stranded DNA (dsDNA) with mismatched 3′ termini [18]. *TREX1* has a critical role in metabolizing endogenous ssDNA and nucleic acids and preventing their accumulation in the cytosol. These can act as danger signals through nucleic acid-sensing pathways, mimicking endogenous retro-elements from retroviral infection, and trigger type 1 interferon innate immune response [19]. The aspartic acid residue at position 18 (p.D18) is located at the enzyme's core active site contributing to DNA binding and is required for its catalytic function [20]. Homozygous missense mutations in the catalytic core (p.D18N, p.D200N, p.D200H) fully suppress enzyme activity (160,000 fold) [21]. In patients with heterozygous mutations, a combination of TREX1 dimers is produced with either normal ($TREX1^{WT/WT}$), decreased ($TREX1^{WT/D18N}$), or fully suppressed ($TREX^{D18N/D18N}$) activity [21]. The ensuing intracellular accumulation of ssDNA leads to constant activation of interferon regulatory factor 3 (IRF3) which can, in the worst cases, lead to a severe auto-inflammatory disorder of the brain and skin with autosomal recessive transmission known as Aicardi-Goutières syndrome (AGS) [22]. Heterozygous mutations in TREX1 have typically been associated with autosomal dominant familial CHLE [8–16], although cases of AGS have also been reported in some patients with the heterozygous D18N mutation [12, 23].

Like CF, CHLE presents with acrocyanosis. The classical definition of CHLE requires two major criteria ((1) cold-induced lesions and (2) skin biopsies compatible with lupus erythematous (LE)) and at least one minor criteria ((1) systemic LE, (2) discoid LE, (3) response to anti-LE therapy, or (4) absence of cryoprecipitate), although it is generally accepted that the last minor criteria is required for diagnosis [24]. Because of the absence of lupus manifestations and the presence of cryoprecipitate in our family, a diagnosis of CHLE had initially been ruled out based on clinical grounds.

When comparing familial reports of CF and CHLE, the clinical description of cold-induced lesions following an autosomal dominant inheritance pattern is extremely similar (Table 1). Skin biopsy results are also comparable and

Table 2 Summary of families previously reported with familial chilblain lupus or primary familial cryofibrinogenemia. ANA, anti-nuclear antibody; BM, basal membrane; Cold agg, cold agglutinin; CryoG, cryoglobulin; CryoF, cryofibrinogen; ssDNA, single-stranded DNA

Source	*n*	Mutation	Clinical manifestations	Skin biopsy findings	Immunologic findings
Familial chilblain lupus families					
Lee-Kirsch MA et al. [8]	18	D18N	Skin: Acrocyanosis Systemic: Arthritis	Perivascular lymphocytic infiltrate Vacuolar interface dermatitis Immune complex on BM	Positive: ANA, ssDNA, anticardiolipin Negative: for CryoF and cryoG
Sugiura K et al. [10]	6	P132A	Skin: Acrocyanosis with ulcers; Systemic: Arthritis	Perivascular lymphocytic infiltrate	Positive: ANA, CryoF not tested
Tüngler V et al. [11]	4	D18N	Skin: Acrocyanosis + ulcers; photosensitive rash Systemic: AGS	Vacuolar interface dermatitis	Negative: ANA and cryoG CryoF not tested
Abe J et al. [12]	16	D18N	Skin: Acrocyanosis Systemic: AGS	Not documented	Negative: ANA, CryoG, Cold agg CryoF not tested
Gunther C et al. [13]	3	D18N	Skin: Acrocyanosis + ulcers Systemic: Arthritis	Vacuolar interface dermatitis Perivascular lymphohistiocytic infiltrate	Positive: ANA, Negative: cryoG CryoF not tested
Yamashiro K et al. [14]	5	D18N	Skin: Acrocyanosis + gangrene Systemic: Cerebral vasculopathy	Leukocytoclastic vasculitis (Small vessel angiitis)	Positive: ANA, Negative: cryoG CryoF not tested
Gunther C et al. [15]	4	H195Q	Skin: Acrocyanosis Systemic: Arthritis	Perivascular lymphocytic infiltrate	Positive: ANA, CryoF not tested
Rice GI et al. [9]	3	G126 fs	Skin: Acrocyanosis + ulcers; Systemic: AGS, arthritis	Not documented	Positive: ANA, Negative: CryoG, Cold agg. and CryoF
Kisla Ekinci RM et al. [16]	2	R114C	Skin: Acrocyanosis + ulcers; Systemic: Cerebral vasculopathy	Not documented	Negative: ANA, ssDNA, cryoG, cryoF
Families initially reported as primary familial cryofibrinogenemia					
Lolin Y et al. [17]	11	?	Skin: Acrocyanosis Systemic: Myalgia, vomiting, and hematuria post-operatively	Not performed	Positive: CryoF Negative: CryoG, Cold agg ANA not tested
Wulffraat N et al. [7]	9	?	Skin: Acrocyanosis Systemic: Fever and arthralgia following cold exposure	Leukocytoclastic vasculitis, Endothelial immune complex depositions	Positive: CryoF Negative: ANA, CryoG
Van Geest AJ et al. [6]	3	?	Skin: Acrocyanosis + ulcers and necrosis	Perivascular lymphohistiocytic infiltrate	Positive: CryoF Negative: cryoG ANA not tested
Current cohort	34	D18N	Skin: Acrocyanosis + ulcers	Perivascular lymphohistiocytic infiltrate	Positive: CryoF Negative: ANA, CryoG

lupus-specific findings of vacuolar interface dermatitis or immune complexes observed in only a minority of cases. The presence of autoimmune antibodies was also inconsistent. Conversely, the presence of cryofibrinogen was excluded in only two reports of familial CHLE.

The discordant findings of lupic sensitization in *TREX1* deficiency suggest that it may be a consequence rather than then a mechanism of the disease. As mentioned earlier, *TREX1*-related disease is caused by the activation of IRF3 [22]. STING-associated vasculopathy with onset in infancy (SAVI), another auto-inflammatory disease caused by the constitutional activation of IRF3, also presents as vessel vasculitis and microangiopathic thrombosis with ulcerative rash on acral surfaces [25]. Autoantibody production in SAVI patients is also highly variable and independent of disease severity further supporting the view that lupic sensitization is a secondary feature that is not required for the syndrome to manifest. In both SAVI- and *TREX1*-related disease, the increased risk for secondary lupus manifestations is likely modulated by other genetic and environmental factors, thus explaining lower occurrence in some families. Interestingly, there is at least one report of SAVI patients who tested positive for CF [26].

The finding of cryofibrinogen in *TREX1*-deficient patients could also be an epiphenomenon of IRF3 activation. Cryofibrinogen consists mainly of fibrinogen, fibrin, and fibronectin and the exact mechanisms for its formation is currently unknown [27]. With *TREX1*-related disease and SAVI, it appears to occur as a non-specific activation of the innate

response. Such a view of CF as an acute-phase reactant is in accordance with its secondary formation with infections or malignancy. It is worth noting that a meta-analysis of 34 genome-wide association studies identified the *IRF3* gene loci as a significant determinant of fibrinogen levels, providing further evidence of a relationship between the two pathways [28].

Affected subjects from our cohort exhibited another very rare missense variant in *COL7A1*, a gene associated with dystrophic epidermolysis bullosa (DEB) [29]. This specific mutation has never been reported in DEB before and none of the patients in our cohort reported the classical signs of skin fragility. The cosegregation of *TREX1* and *COL7A1* variations is thus most likely explained by physical proximity on the same chromosome (100 kb).

One limitation in this study is that cryofibrinogen was not measured in all affected members of the family as the majority of blood sampling was done in rural Quebec where these analyses are not readily available. For that reason, the prevalence of CF in *TREX1*-related disease cannot be ascertained.

Conclusion

In conclusion, we have described the largest familial cohort of *TREX1*-related disease with 34 affected individuals. Both index cases presented with CF without any personal or familial history lupus manifestations, in line with a diagnosis of primary familial CF. This study and a review of previous reports on familial CF and CHLE leads us to conclude that the spectrum of *TREX1*-related disease should be broadened and the diagnosis sought in all patients presenting with a familial history of cold-induced acrocyanosis regardless of the presence or absence of lupus manifestations or cryoprecipitates.

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Authors' Contributions Dr. Bégin had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Cossette, Bégin.

Acquisition of data: Paradis, Des Roches, Leclerc, Meloche, Gravel, Cadieux-Dion, Cossette, Bégin.

Analysis and interpretation of data: Cadieux-Dion, Paradis, Bégin.

Drafting of the manuscript: Cadieux-Dion, Paradis, Bégin.

Critical revision of the manuscript for important intellectual content: All.

Administrative, technical, or material support: Meloche, Gravel.

Study supervision: Cossette, Bégin.

Compliance with Ethical Standards

This project was approved by the research ethics committee of the Centre Hospitalier de l'Université de Montreal. The participants received no financial compensation.

Conflict of Interest The authors declare that they have no conflict of interest.

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