G908R NOD2 variant in a family with sarcoidosis

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Abstract

Background: Sarcoidosis is a systemic disease characterized by the formation of immune granulomas in various organs, mainly the lungs and the lymphatic system. Exaggerated granulomatous reaction might be triggered in response to unidentified antigens in individuals with genetic susceptibility. The present study aimed to determine the genetic variants implicated in a familial case of sarcoidosis.

Methods: Sarcoidosis presentation and history, NOD2 profile, NF-κB and cytokine production in blood monocytes/macrophages were evaluated in individuals from a family with late appearance of sarcoidosis.

Results: In the present study, we report a case of familial sarcoidosis with typical thoracic sarcoidosis and carrying the NOD2 2722G > C variant. This variant is associated with the presence of three additional SNPs for the IL17RA, KALRN and EPHA2 genes, which discriminate patients expressing the disease from others. Despite a decrease in NF-κB activity, IL-8 and TNF-A mRNA levels were increased at baseline and in stimulated conditions.

Conclusions: Combination of polymorphisms in the NOD2, IL17RA, EPHA2 and KALRN genes could play a significant role in the development of sarcoidosis by maintaining a chronic pro-inflammatory status in macrophages.

Keywords: Familial sarcoidosis, NOD2, Genetic susceptibility

Background

Sarcoidosis is a systemic granulomatous disease of unknown cause. A genetic predisposition is suggested by familial histories and the risk for the disease multiplied by 80 in monozygotic twins of index cases [1, 2]. Genome-wide association studies and candidate gene-driven investigations identified several genetic risk foci for sarcoidosis, including butyrophilin-like 2 gene [3–5], Annexin 11 [6], a locus on chromosome 11q13.1 [7], several loci in the HLA region [8] and in the IL23/Th17 signalling [9]. NOD2 mutations have been evidenced in granulomatous diseases, including Blau syndrome (BS), early onset sarcoidosis (EOS) and Crohn’s disease (CD). NOD2 is a cytosolic protein involved in sensing microbial cell wall components and regulating inflammatory processes by activating NF-κB [10]. A deficit in sensing bacteria in monocytes/macrophages might result in an exaggerated inflammatory response by the adaptive immune system. Thus NOD2 mutations are considered to play a major role in the pathogenesis of granulomatous diseases [11, 12]. Variants may concern different NOD2 domains, NACHT domain for BS and EOS and LRR domain for CD [11, 13]. NACHT domain variants enhanced NOD2 activity, whereas LRR domain variants reduced NOD2.

Although scientific evidence was provided indicating that the NOD2 signalling pathway was involved in sarcoidosis pathogenesis, selection of patients with confirmed sarcoidosis from the Sarcoidosis Genetic Analysis study population [14] and the ACCESS study [15] with or without joint and skin involvement showed no evidence of NOD2 mutation [16]. Up to now, no study has evidenced any NOD2 mutation in sarcoidosis with a late typical presentation. The role of the NOD2 gene in sarcoidosis was associated with increase susceptibility for developing sarcoidosis [17, 18].

The SARCFAM project is a French national project on familial sarcoidosis which allowed the recruitment of more than 180 families with at least two first-degree affected members [19]. Screening of nine sarcoidosis by WES has provided extensive and complex data which
are included in a larger program of the SARCFCAM project. Among this series, a subset of 10 families gathered more than 3 patients. One of them referred as “X” was
of particular interest due to the identification of NOD2 variants, including the G908R mutation that has been described in Crohn’s disease. Identification of the NOD2 variant in the LRR domain (G908R in exon 8) was confirmed by Sanger sequencing.

In the present study, we report sarcoidosis presentation and history, NOD2 profile and NF-κB and cytokine production in blood monocytes in affected patients of the family X and to compare genetic and NF-κB and cytokine profiles with members of the family unaffected by sarcoidosis and safe controls.

Methods
Populations
The study received institutional review board approval (CPP IRN number 00009118) according to French legislation. Written informed consent for all participants was obtained for genetic and biological investigations. Three groups were studied:

Members of family X with confirmed sarcoidosis (n = 4) (Table 1 and Fig. 1). Sarcoidosis diagnosis was made according to sarcoidosis statement [20]. Three of them could be investigated (IB, IIA and IIB). In them, clinical presentation was typical, evidence of non-caseating granulomas was obtained and there was no element in favour of any alternative granulomatous disease. None of them presented any element in favour of BS, EOS nor CD. In particular, there was no digestive tract history except for one patient with colic polyps who underwent twice colonoscopy without evidence of macroscopic or histologic arguments for CD.

Members of family X unaffected by sarcoidosis (n = 2) (Fig. 1). Two members of the family (IIC and IID), who were brother or sister of two patients, son or daughter of one patient and nephew or niece of the fourth patient, with no history of sarcoidosis were investigated in 2016. For both, clinical examination, chest X ray and blood biology including serum angiotensin were normal.

Control volunteers (n = 4): Four normal volunteers, aged 69, 65, 48 and 53 with no history of sarcoidosis nor any known current disease served as normal controls.

Genetic testing
Blood DNAs were obtained from three patients and tested for NOD2 exon 8, IL17RA exon 11, KALRN exon 1 and EPHA2 exon 17 sequence variants. PCR reaction was performed as described in the Additional file 1.

Targeted exome sequencing
Genomic DNA was captured using Agilent in-solution enrichment methodology (SureSelect Human Clinical Research Exome, Agilent) with the supplied biotinylated oligonucleotides probes library (Human Clinical Research Exome, Agilent), followed by paired-end 75 bases massively parallel sequencing on Illumina HiSEQ 4000. The DNA library was prepared as described in the Additional file 1.

Screening of SNP variants identified by WES
SNP variants identified in family X upon WES screening were analyzed for a putative functional effect on the protein by using the SIFT and Polyphen2 softwares (see in the Additional file 1). The minor allele frequencies (MAF) were evaluated by the ExAC online database (see in the Additional file 1, Bioinformatics and Statistical and functional evaluation of variants in silico). A MAF less than 0.01 suggest a rare variant and not a common polymorphism.

Peripheral blood mononuclear cells isolation, culture and stimulations
Twenty mL of total blood sampled in EDTA tubes for routine hematologic analysis were collected. Peripheral blood mononuclear cells (PBMCs) were isolated and differentiated in macrophages as described in the Additional file 1. Macrophages were then stimulated with either saline serum (Ctrl), or 10 μg/mL of N-Acetylmuramyl-L-alanyl-D-isoglutamine hydrate (MDP, muramyl dipeptide) (Sigma-Aldrich France), or 1 μg/ml Lipopolysaccharides from Escherichia coli 0111: B4 (LPS) (Sigma-Aldrich) for 1 h for the NF-κB activation study or 24 h for the transcriptional study.

NF-κB activation
Activation of the different NF-κB family members (p50, p52, p65, c-Rel and RelB) was assayed using the TransAM® NF-κB Family kit (Active Motif, Belgium) according to the manufacturer’s protocol.

| Table 1 | Clinical characteristics of family X members |
|---------|--------------------------------------------|
| Family member | Year of birth | Sex | Age at sarcoidosis diagnosis | Chest X ray stage | Extra-thoracic sarcoidosis | Sarcoidosis duration (years) | Sarcoidosis treatment |
| IIA | 1955 | M | 35 | 4 | PH | 28 | corticosteroid |
| IIB | 1957 | M | 51 | 1 | spinal cord | 10 | corticosteroid/cyclphosphamid |

M male, F female; *according to (17), PH pulmonary hypertension under specific vasodilator treatment
RNA extraction and RT-QPCR
RNA were prepared as described in Additional file 1. Quantitative PCRs were performed in presence of Absolute QPCR Mix (Thermo Fisher Scientific) with primer sets specific to TNF-A, IL-8, IL-6, NOD2 and IL17RA (see in the Additional file 1: Table S1).

Results
Sarcoidosis presentation, outcome and treatment
Sarcoidosis presentation, outcome and treatment are shown in Table 1. Sarcoidosis was diagnosed in 35, 41, 43 and 51-year-old patients. Typical bilateral hilar lymphadenopathy was evidenced on thorax imaging in all four cases with lung infiltration in two cases. Bilateral upper lobes fibrosis was seen in two cases, associated with pulmonary hypertension in only one case. Other manifestations were respectively skin lesions for I and spinal cord localization for II. In three cases (IB, IIA, IIB), there was a prolonged course (respectively 35, 27 and 9 years) with no healed case till now. Patient IB with a well-tolerated chronic sarcoidosis has upper lobes pulmonary fibrosis with calcification of hilar and mediastinal lymph nodes. She received no systemic therapy for sarcoidosis, except treatment for recurrent infectious exacerbations. The exact duration of the disease course is unknown. For IIA, a corticosteroid treatment was given after 20 years due to lung progression and occurrence of lung fibrosis. There was a good response with glucocorticoids which could be tapered until 7 mg/d with no evidence of residual activity on recent investigations. Eventually, for IIB, because of spinal cord involvement, a treatment associating high doses of corticosteroids and cyclophosphamide was given with a favourable response. Now, treatment consists in low doses of prednisone without relapse.

NOD2 investigation
The NOD2 variant (SNP - Ggc/Cgc – rs2066845 – position chr.16.50756540 – transcript NM_022162) identified in this family is a missense mutation previously described in the context of Crohn’s disease. It consists of a G908R substitution located in exon 8. The minor allele frequency as suggested by the ExAC database is 0.009917 (http://exac.broadinstitute.org/). In family X, this variant was found in the three patients with sarcoidosis and one among two offsprings free of the disease. No control had NOD2 mutation. In addition, selective extraction of rare variants from WES data allowed us to identify three other single nucleotide variants in the three patients with sarcoidosis. These SNPs target the coding regions of the IL17RA, KALRN and EPHA2.
genes, causing either missense (IL17RA, EPHA2) or nonsense (KALRN) variants. Data on the various SNPs are summarized in Table 2. In the series of 9 families screened by WES and including family X, a total of 7 deleterious and/or rare variants was observed in the three genes, either EPHA2 (3 variants in 3 families), KALRN (2 variants in 2 families) and IL17RA (2 variants in 2 families) (Table 3). The rs139787163 variant of EPHA2 (p.Ala951Thr) was observed two times despite a very low MAF value (ExAC: 0.000346).

**NFκB activation is reduced in sarcoidosis patients with NOD2 G208R mutation**

Activation of NOD2 promotes its binding to RIP2 kinase, triggering the complex activation and initiating the activation of multiple complexes, including MAP kinases and IkB kinases [21]. This activation allowed the release of active NF-κB/Rel complexes that translocate to the nucleus where they induce target gene expression involved in innate and adaptive immunity, inflammation, stress responses, B-cell development, and lymphoid organogenesis. In the present study, activation of different NF-κB family members (p50, p52, p65, c-Rel and RelB) was assayed in monocytes and macrophages in basal condition or in response to either MDP (specific NOD2 activator) or LPS from control subjects or family members. NF-κB p50 and p65 activations were increased in response to MDP and LPS stimulation in both control subjects and family patients, except the patient IB whose macrophages poorly responded to both treatments (Fig. 2a, b). Similarly, NF-κB p52 activation was less in macrophages from patient IB (Fig. 2c). No major differences were observed in NF-κB cRel and RelB activation in macrophages from control subjects or family members in stimulated by MDP or LPS (Fig. 2d, e).

To determine whether reduced NF-κB activation in untreated NOD2 G208R sarcoidosis patient (IB) decreases inflammatory cytokines expression, mRNA levels for IL-8, TNF-A, IL-6 genes were assessed by quantitative RT-PCR (qRT-PCR) in monocytes and macrophages in basal condition or in response to either MDP or LPS from control subjects or family members. Strikingly, both IL-8 and TNF-A mRNAs were expressed at high levels in macrophages from patient IB in basal and stimulated conditions compared to other family members and control subjects (Fig. 3a, b). By contrast, IL-6 mRNA levels were similar in both groups in each culture conditions (Fig. 3c). In addition, NOD2 mRNA levels were increased in patient IB in basal condition and in response to MDP (Fig. 3d). By contrast, IL17RA were similar in both groups in each culture conditions (Fig. 3e).

**Table 2** Description of heterozygous variants identified by whole-exome-sequencing in the family X

| Gene variant | NOD2 2722G > C exon 8 NM_022162 | IL17RA 958 T > C exon 11 NM_014339.6 | KALRN 28C > T exon 1 NM_007064.3 | EPHA2 2875G > A exon 17 NM_004431.3 |
|--------------|---------------------------------|----------------------------------------|---------------------------------|----------------------------------|
| Chr.         | 16                              | 22                                     | 3                               | 1                                |
| Position     | 50,756,540                      | 17,586,757                             | 124,303,696                     | 16,451,766                       |
| QUAL         | 528                             | 3844                                   | 4578                            | 10,401                           |
| Deph         | 528                             | 3844                                   | 4578                            | 10,401                           |
| rs ID number | rs2066845                       | rs140221307                            | rs56407180                      | rs139787163                      |
| Single nucleotide variant | missense | missense | nonsense | nonsense |
| PolyPhen2 prediction | 0.986   | 0.972a       | STOPa      | STOPa       |
| SIFT         | 0.01                            | 0a                                     | STOPa                            | STOPa                           |
| ExAC global MAF | 0.009917 | 0.001801   | 0.002382  | 0.00346  |
| Protein      | NOD2 G908R                      | IL17RA W320R –                         | –                               | EPHA2 A959T                      |
| Prior associations with disease | Crohn’s disease, Psoriatic arthritis, Blau syndrome | Familial Candidiasis – | Age-related cortical cataract |
| Patient IB   | Het                             | Het                                    | Het                             | Het                              |
| Patient IIA  | Het                             | Het                                    | Het                             | Het                              |
| Patient IIB  | Het                             | Het                                    | Het                             | Het                              |
| Patient IIC  | Het                             | A                                      | A                               | A                                |
| Patient IID  | A                               | A                                      | A                               | A                                |

Het heterozygous, A minor allele (C) absent, Chr chromosome, SNP single nucleotide polymorphism; Depth represents the number of reads identifying the SNP variant. QUAL, a quality parameter measuring the probability p that the observation of the variant is due to chance (for ex: QUAL = n, p = 1/n). It includes the DEPTH parameter and the coverage of the genomic sequence. The in silico functional evaluation of SNP variants was performed by using bioinformatics softwares SIFT, PolyPhen-2. The value indicating a putative pathogenic effect are near of 0 for SIFT and near of 1 for Polyphen2, as indicated by the a. The minor allele frequency of SNP variants were evaluated by using the ExAC online database (http://exac.broadinstitute.org/). A global MAF including all ethnic origins lower than 0.01 suggest a rare variation and not a common polymorphism.
**Table 3** Variants identified in other SARCFAM families

| GENE  | rs     | Exon | Codon | TYPE                | EFFECT     | SIFT   | POLYPHEN | Accession       | Number of family | MAF (ExAC) | NUCLEOTIDE | CHR | POSITION | DEPTH mini |
|-------|--------|------|-------|---------------------|------------|--------|----------|----------------|-----------------|-------------|------------|------|----------|------------|
| EPHA2 | rs139787163 | 3    | 959   | missense           | Ala959Thr  | 0.05   | 0.828    | NM_004431.3     | Family X        | 0.000346    | Gcc/Acc    | 1    | 16,451,766 | 48         |
| EPHA2 | rs150790360  | 3    | 165   | missense           | Val165met  | 0      | 0.062    | NM_004431     | 1               | 0.001535    | Gtg/Atg    | 1    | 16,475,203 | 157        |
| EPHA2 | rs139787163 | 17   | 959   | missense           | Ala959Thr  | 0.05   | 0.828    | NM_004431     | 1               | 0.000346    | Gcc/Acc    | 1    | 16,451,766 | 48         |
| KALRN  | rs56407180  | 1    | 10    | nonsense           | Arg10ter   | STOP   | GAIN     | NM_007064.3     | Family X        | 0.002382    | c.28C > T  | 3    | 124,303,696 | 528        |
| KALRN  | rs35298864  | 38   | 1930  | missense           | Arg1930Met | 0      | 0.891    | NM_001024660  | 1               | 0.007276    | aGg/aTg    | 3    | 124,369,782 | 178        |
| IL17RA | rs40221307  | 11   | 320   | missense           | Trp320Arg  | 0      | 0.972    | NM_01433996    | Family X        | 0.001801    | c.958 T > C | 22   | 17,586,757 | 370        |
| IL17RA | rs41323645  | 13   | 691   | missense           | Ala691Thr  | 0.35   | 0.926    | NM_01433996    | 1               | 0.3023      | c.2071G > A | 22   | 17,590,180 | 160        |
| IL17RB | rs2232346   | 9    | 278   | missense           | Phe278Leu  | 0.65   | 0        | NM_0187253     | Family X        | 0.001915    | c.832 T > C | 3    | 53,892,830 | 141        |
| IL17RC | nd      | 6    | nd    | splice donor       | frameshift | splicing | defect    | 1               | nd             | c.790 + 1G > T | 3    | 9,962,287  | 155        |

Additional informations on IL17R
Discussion

To our knowledge, this is the first time that the NOD2 2722G > C (rs2066845) variant substituting a Glycine to Arginine at codon 908 in exon 8 in the leucine rich repeat (LRR) domain of the NOD2 protein is reported in a case of familial sarcoidosis with late and typical presentation of the disease. This variant was observed in an unaffected relative, suggesting that other genetic and/or epigenetic factors may contribute to the occurrence of the disease. Indeed, WES analysis discriminated affected versus unaffected individuals by three putative pathogenic SNP variants in the IL17RA, KALRN and EPHA2 genes.

NOD2 is essential in regulating both inflammatory and immunologic homeostasis. In silico pathogenic evaluation (p) with the SIFT (p = 0.01) and POLYPHENv2 (PSIC score difference = 2.407) suggest a strong disturbing effect on the primary amino acid sequence raising the question of a significant impact in sarcoidosis pathogenesis. The role of the G908R mutation of the NOD2 gene in sarcoidosis is controversial either with

Fig. 2 NF-κB activation is decreased in untreated NOD2 G208R sarcoidosis patient. NFκB activation (p50 (a), p65 (b), p52 (c), RelB (d), cRel (e)) (measured as OD450 nm) was assessed by TransAM NFκB kit (Active Motif) in control (C) and family X (F) monocytes (Mono) or monocytes derived macrophages (MØ) in basal condition (Ctrl) or treated with MDP or LPS for 1 h.
increase susceptibility for developing sarcoidosis [17, 18] or a minor role of the 2722G > C variant in the pathogenesis of sarcoidosis [22–24]. In the present case of familial sarcoidosis, 3 out of 4 patients with the variant 2722G > C developed sarcoidosis, while the other unaffected offspring did not carry the variant. Observation of a family member with NOD2 variant but no sarcoidosis is similar to the numerous individuals free of any disease, particularly of CD despite carrying the same variant, indicating that gene/environmental interactions and possibly gene/gene interactions (epistasis) are also essential [25]. Such association of sarcoidosis with the NOD2 2722G > C variant is certainly a very rare finding. It was not observed in Bello’s paper focused on adult patients with sarcoidosis involving eyes, skin and joints as in EOS and BS [16]. In our nine other families with at least 3 members affected, none was associated to any NOD2 variant. Among our family collection, families with 3 or more affected members (n = 10) represent probably a small subset among sarcoidosis families with probably specific genetic predisposal or shared environmental factors.

It is of particular interest to observe the NOD2 2722G > C variant associated with a very typical presentation of sarcoidosis. The up-regulation of IL-8 and TNF-A expression in untreated NOD2 G208R sarcoidosis patient is shown in Fig. 3. IL-8 (a), TNF-A (b), IL-6 (c), NOD2 (d) and IL17RA (e) mRNA levels were assessed by RT-qPCR in control (C) and family X (F) monocytes (Mono) or monocytes derived macrophages (MØ) in basal condition (Ctrl) or treated with MDP or LPS for 24 h.
sarcoidosis, all patients showing bilateral hilar lymphadenopathy and lymphatic distribution of lung micronodules distinct from EOS when lung involvement was present [26]. By contrast evidence of lung infiltration is very rare in EOS with a very different radiologic pattern and no lymphadenopathy [11]. Another question is the impact of NOD2 variant on the outcome which was characterized by a very long course in all patients, a condition usual in CD and perhaps associated to persistent abnormalities of microbiota induced by the variant. Indeed, deletion of Nod2 in mice reduced bacterial activity in the gut, promoting an increased susceptibility to colonization by both the commensal microbiota and pathogenic bacteria, thus triggering intestinal inflammation [27]. In addition, Nod2 deletion impairs autophagy in macrophages allowing intracellular survival of bacteria delaying bacterial clearance [28]. In our family, any confusion with CD either as main diagnosis or as comorbidity was ruled out by the absence of suggestive history of digestive tract disease particularly in one patient who had two colonoscopic examinations.

Both gain of function NOD2 mutant alleles like in BS and EOS and loss of function NOD2 mutant alleles like in most common CD and probably in our family with sarcoidosis might induce autoinflammatory disorders [29, 30]. During sarcoidosis, NF-κB is activated in site of organ involved [31–33]. In BS and EOS, gain of function NOD2 variants are associated with NF-κB activation. By contrast, we observed NFκB downregulation in our untreated patient as in CD patients with the NOD2 2722G > C variant. These data are supported by in vitro experiments showing that NFκB activity is reduced at baseline and in response to MDP in HEK293T cells expressing the NOD2 2722G > C variant [34–36]. Interestingly, NF-κB activity was upregulated in response to MDP in family X treated patients as well as the unaffected carrier of the variant, similarly to normal controls suggesting that treatments efficient against sarcoidosis activity may restore NF-κB activity altered by the presence of the NOD2 2722G > C variant. Indeed, patients treated by glucocorticoids responded well to the treatment, indicating that they were not refractory to corticoids [37] by comparison to Crohn disease patients [38]. Although increased NF-κB activity in patients with corticoids is obviously in conflict with what could be expected with anti-inflammatory drugs, previous studies showed that glucocorticoids could potentially contribute to pro-inflammatory activation by inducing the expression of the Macrophage migration inhibitory factor [39]. By contrast, in response to LPS stimulation, NF-κB activity was maintained in the untreated patient IB to similar levels observed in family X treated patients and normal controls, indicating that stimulation of NF-κB through NOD2- independent pathways remains possible. NF-κB is an important transcription factor for genes encoding cytokines like IL-8 and TNF-A, this last one being a cornerstone in sarcoidosis pathogenesis. In patient IB, despite a decrease in NF-κB activity, IL-8 and TNF-A mRNA levels were increased at baseline and in stimulated conditions, indicating a chronic pro-inflammatory status in macrophages. As in Crohn disease patients with the NOD2 2722G > C variant, patient IB led to an apparent paradox: reduced NF-κB transactivating activity associated with more inflammation. Recently Strober et al... showed that in mice, activation of NF-κB in response to ligands to toll-like receptor 2 (TLR-2) is down-regulated by NOD2 [40]. In the absence of an efficient form of NOD2 the TLR signaling pathway is maintained, leading to increased production of inflammatory cytokines and the development of pathogenesis. Other data suggest that virulence and pathogens factors may trigger the NOD1/NOD2 signaling pathway indirectly by activating Rac1 [41]. Rac1 has been shown to upregulate NF-κB activity in a PAK1 (p21-activated kinase) dependent manner by stimulating the nuclear translocation of p65 subunit of NF-κB [42]. In addition, Hedl et al showed that acute stimulation of NOD2 triggered in human macrophages the secretion of anti-inflammatory cytokines, including IL-10 and IL1ra [43]. Thus, we could hypothesize that in NOD2 2722G > C patients, an imbalance occurs in favour of a pro-inflammatory phenotype.

One of the major issue was to find any genetic data which might discriminate family X members expressing clinically the disease from the others. Remarkably, in addition to NOD2 2722G > C, three single nucleotide variants for the IL17RA, KALRN and EPHA2 genes were found in the family X patients with sarcoidosis. By comparison, as shown in Table 3, screening of the nine families by WES analysis showed the presence of single deleterious variants in a total of 7 families out of 9, one of them (EPHA2 – rs139787163) being common in two distinct families despite a very low minor allele frequency (MAF = 0.000346).

The IL17RA 958 T > C variant modifies the amino acid sequence in the extracellular domain of the IL17RA, possibly altering its function. IL17RA (interleukin 17A receptor) is capable to form a heterodimer complex to bind both interleukin 17A and IL-25. IL17RA plays a pathogenic role in many inflammatory and autoimmune diseases, including rheumatoid arthritis, psoriasis, Candida albicans infection and Crohn disease [44–46]. Activation of IL17RA leads to induction of expression of inflammatory cytokines such as CXCL1, CXCL8/IL8 and IL6 through the trans-activation of NF-κB, AP-1 and C/EBPβ. Involvement of IL17 signalling was shown in sarcoidosis [47]. Notably, IL-17-producing T cells are increased in peripheral blood and lungs of subjects with sarcoidosis compared with controls [48]. In addition, IL-17A plays a major role in granuloma formation in response to mycobacterial infections in mice [49, 50].
Likewise Th17 cells have been implicated in the development of Crohn’s disease [51]. Notably, McGovern et al. showed that IL17RA genetic variants increased susceptibility to inflammatory bowel disease pathogenesis and demonstrated the cumulative risk of IL17RA variants with genes of the IL23/IL17 pathway in the development of CD [46]. Interestingly, Kurdy et al. demonstrated that IL17 is directly regulated at the transcriptional level by a complex including Rac1, Tiam1 and RORyt (RAR-related orphan receptor gamma), the master transcription and differentiation factor of Th17 cells [52].

The EPHA2 2875G > A variant is located within the conserved cytoplasmic domain of the receptor. This variant was associated with age-related cortical cataract [53–56]. Erythropoietin-producing hepatoma (Eph) receptors are a family of receptor tyrosine kinases that can bind ephrin ligands. EphA receptors are expressed on CD4+ and CD8+ T cells, dendritic cells (DCs), and Langherans cells [57, 58] and are involved in T cell function [59]. Eph receptors are involved in axon guidance, vascularization, tissue assembly, and cell adhesion and migration [60–63]. Khounlothm et al. showed that EphA receptors play a role in the pathogenesis of M. tuberculosis infection by reducing the migration of T cells and DCs to the site of infection, producing an environment that favours bacterial persistence [64]. Activated EPHA2 interacts with Rho family GEF in endosomes and play a role in ephrin-dependant Rac1 activation [65]. Interestingly, variants for EPH receptor family members have been associated with inflammatory bowel diseases. Hafner et al. showed an increased expression of Eph-B2 that increased epithelial cell mobility in the intestinal epithelium of Morbus Crohn patients [66].

The KALRN 28C > T variant is a non-sense variant targeting Kalirin, a multidomain guanine nucleotide exchange factor (GEF) for small GTP-binding proteins of the Rho family. Multiple kalirin isoforms containing different combinations of functional domains are predominantly expressed in brain, except KALRN 9 that is more widely expressed [67]. Kalirin is known to be involved in active remodeling of synapses and dendritic maturation in early development [68]. Kalirin was also shown to play a neuroprotective role during inflammation of the central nervous system by inhibiting iNOS activity [69]. In smooth muscle cells, Kalirin interacts with Rac1, promoting SMC migration and proliferation [70]. Thus, it appears that both EPHA2 and KALRN are implicated in dendritic cell maturation and migration. Dendritic cells have been implicated in sarcoidosis [71]. Mathew et al. showed that myeloids DCs function was reduced in sarcoidosis patients, possibly contributing to susceptibility and persistence of the chronic inflammation [72]. Interestingly in family X, member IIC who carries the NOD2 2722G > C variant only, did not develop sarcoidosis. These data suggest that the NOD2 2722G > C variant was not sufficient to trigger pulmonary sarcoidosis by itself and it is the presence of other variants (IL17RA 958 T > C, EPHA2 2875G > A and KALRN 28C > T) that contribute to the pathology in patients with pulmonary sarcoidosis of family X, perhaps by enhancing the development and chronicity of pulmonary sarcoidosis in family X.

Taken together, our data suggest a functional and pathogenic link between EPHA2, KALRN and IL17RA in the occurrence of the disease of family X sarcoidosis patients, acting in addition to the NOD2 functional defect.

Conclusions
For the first time in the literature, we described the presence of the NOD2 2722G > C variant in a case of familial sarcoidosis. Our finding further establishes that the NOD2 2722G > C variant in combination with variants for IL17RA, EPHA2 and KALRN genes could play a significant role in the development of sarcoidosis. Future functional studies are required to reveal the causal regulatory variation of the various loci and the immunogenetic basis related to sarcoidosis.

Additional file

Additional file 1: Contains supplementary data for the Material and Methods section. (DOC 64 kb)

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Availability of data and materials
All data generated or analysed during this study are included in this published article [and its Additional file 1].

Authors’ contributions
VB designed research studies, contributed to the experiments, analyzed the data and wrote the manuscript. AC designed research studies, contributed to the experiments, analyzed the data. DB analyzed the data and wrote the manuscript. YP designed research studies, contributed to the experiments, analyzed the data and wrote the manuscript. CCA analyzed the data and wrote the manuscript. CP reviewed the manuscript. DV designed research studies, contributed to the experiments, analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study received institutional review board approval (CPP IRN number 00009118) according to French legislation. Written informed consent for all participants was obtained for genetic and biological investigations.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.
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