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| Title            | Heterozygous Mutations in OAS1 Cause Infantile-Onset Pulmonary Alveolar Proteinosis with Hypogammaglobulinemia                                                                                           |
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## **SUPPLEMENTAL NOTE**

### **CASE REPORT**

We found five individuals (A-II-1, A-II-3, A-II-4, B-II-1, and C-II-1) with infantile-onset pulmonary alveolar proteinosis (PAP) with hypogammaglobulinemia from three unrelated families (Figure S1 and Table 1).

[A-II-1], male

This male infant was born to non-consanguineous parents (Figure S1A and Table 1). His clinical course was reported previously.<sup>1</sup> Briefly, he did not show respiratory problem at birth, but died at 91 days of age following sucking difficulty since 9 days of age, hospitalization with a diagnosis of pneumonia at 39 days of age, and progressive respiratory failure requiring mechanical ventilation since 55 days of age. Surfactant replacement was not effective for his respiratory failure. Autopsy revealed PAP, but the cause of PAP was unknown.

[A-II-2], male

He was healthy (Figure S1A).

[A-II-3], male

This male infant showed no respiratory problems at birth, but was hospitalized for fever, cough, and failure to thrive at 39 days of age with a diagnosis of pneumonia (Figure

S1A and Table 1). A diagnosis of PAP was made after bronchoalveolar lavage (BAL) examination at 100 days of age following supplemental oxygen requirement since 86 days and mechanical ventilation since 92 days of age. Steroid administration, inhalation of nitric oxide, and extracorporeal membrane oxygenation were ineffective, and he finally died of respiratory failure at 163 days of age before bone marrow engraftment following hematopoietic stem cell transplantation (HSCT) with his father's bone marrow at 151 days of age. Autopsy confirmed PAP and indicated cytomegalovirus infection in the lung. Cytomegalovirus infection was considered as a complication at the end stage of his clinical course. No abnormalities in *GMCSF*, the gene encoding GM-CSF, were detected in genomic DNA from his peripheral white blood cells (WBCs). Surfactant protein (SP)-B was detectable in the BAL fluid. No significant anti-GM-CSF antibody was detected in freeze-stored BAL.

[A-II-4], female

The clinical course before 5 years of age (Figure S1A and Table 1) was reported previously as GPAP with hypogammaglobulinemia.<sup>2</sup> Briefly, similar to the two elder brothers affected by GPAP, this female infant did not have respiratory symptoms at birth. However, she became febrile (40°C) unresponsive to antibiotics and had generalized skin rash at 23 days of age. Abnormal chest X-ray and CT findings were first observed at 42 days of age as shown in Figures S2A and S3A, respectively. Her respiratory dysfunction was repeatedly responsive to monthly intravenous

immunoglobulin (IVIG) administration. She required hospitalization 19 times over 10 years from 1 to 10 years of age for treatment of recurrent infections, mainly by viruses, including pneumonia, bronchitis, gastroenteritis, peritonitis, otitis media, and cystitis. She showed excessively strong reaction to each infection. Although IVIG was effective to improve respiratory function and overcome infection, she required home oxygen therapy since 8 years of age (Figure S3B). Finally, she died of respiratory failure at 11.3 years of age while awaiting HSCT (Figure S2C). Autopsy showed alveoli diffusely occupied with PAS-positive materials and small and non-foamy alveolar macrophages (AMs) (Figures S5A–D).

Her serum levels of SP-A (up to 154 ng/mL), Krebs von den Lungen-6 (KL-6) (up to 46190 U/mL), IgM (up to 60 mg/dL), and IgA (up to 57 mg/mL) were elevated at 11 years old. SP-D level was consistently within the normal range during the course. The serum IgG level showed a trough of 102 mg/dL at 102 days of age, and WBC count was consistently high, ranging from 10000 to 86000 / $\mu$ L throughout her clinical course. Splenomegaly and proteinuria were observed before monthly IVIG but disappeared after initiation of this therapy. BAL fluid (Figure S4A) contained acidophilic sediments that were stained strongly with PE10 (anti-SP-A antibody), high levels of SP-A (10  $\mu$ g/mL; reference 1.3–5.2  $\mu$ g/mL) and SP-B (700 ng/mL) with normal SP-A:SP-B ratio, GM-CSF (17.7 pg/mL), and macrophage colony stimulating factor (M-CSF) (70840 pg/mL). BAL fluid contained large number of small and non-foamy AMs (Figure S4B). The AMs showed weak staining for PE10 and CD14 but

were quite different from large and foamy AMs observed in individuals with autoimmune PAP (APAP). Her AMs cultured in medium even with GM-CSF died within a short time. No significant anti-GM-CSF antibodies were detected in her sera

[B-II-1], female

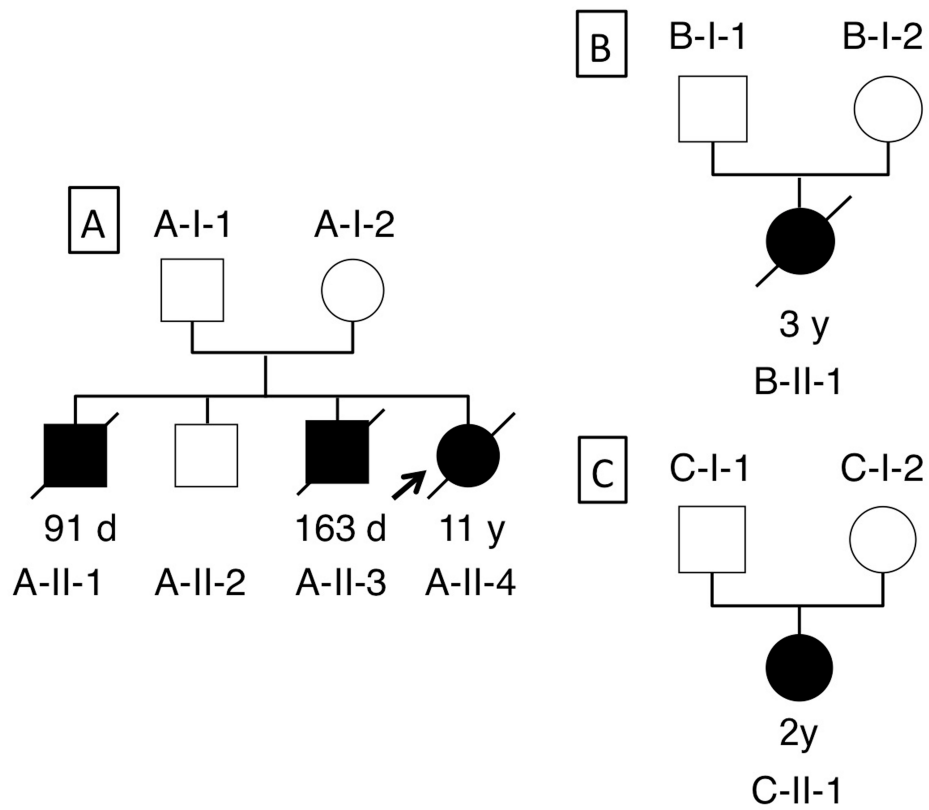
The clinical course of this female infant was reported previously.<sup>3</sup> Briefly, she was born with no respiratory symptoms, but was hospitalized due to vesicles on the face, buttocks, and extremities at 29 days of age (Figure S1B and Table 1). Leukocytosis and low serum levels of IgG (101 mg/dL), IgM (3 mg/dL), and IgA (2 mg/dL) were observed on admission. She then presented with tachypnea without cough at 2 months of age. Confluent consolidations were seen in both lungs on chest CT at the age of 4 months (Figure S3C). The findings of BAL fluid were consistent with the diagnosis of PAP (Figure S4C). As AM dysfunction was considered, HSCT with cord blood was performed at 8 months of age.<sup>3</sup> Respiratory function improved markedly 21 days after myeloid engraftment followed by no dense consolidations on chest CT 2 months after HSCT (Figure S3D). Although she had no recurrence of respiratory dysfunction, she was complicated by reactivated cytomegalovirus infection and finally died from renal failure with histological findings of focal glomerulosclerosis at 3 years of age.

[C-II-1], female

The clinical course of this female infant was also reported previously.<sup>3</sup> Briefly, she was

born with no respiratory symptoms, but was hospitalized at 5 months of age for pneumonia caused by respiratory syncytial virus (RSV) requiring mechanical ventilation for 10 days (Figure S1C and Table 1). In addition, she suffered from cytomegalovirus and subsequently coronavirus NL63 infection. She showed low serum levels of IgG (103 mg/dL), IgM (11 mg/dL), and IgA (3 mg/dL). Although sequential IVIG and subcutaneous immunoglobulin appeared to be effective, she had persistent respiratory symptoms and progressive consolidation on chest X-ray and CT (Figures S2D and S3E). Anti-GM-CSF antibody was not detected in the serum. BAL fluid at 8 months of age contained PAS-positive acidophilic material (Figure S4D), small and non-foamy AMs (Figure S4E), and elevated levels of SP-A ( $> 2 \mu\text{g/mL}$ ) and SP-D (8240 ng/mL). These findings were consistent with PAP. HSCT with cord blood performed at 11 months of age was effective. She left hospital with no respiratory symptoms at 16 months of age (Figure S3F) after overcoming graft versus host disease.

## SUPPLEMENTAL FIGURES



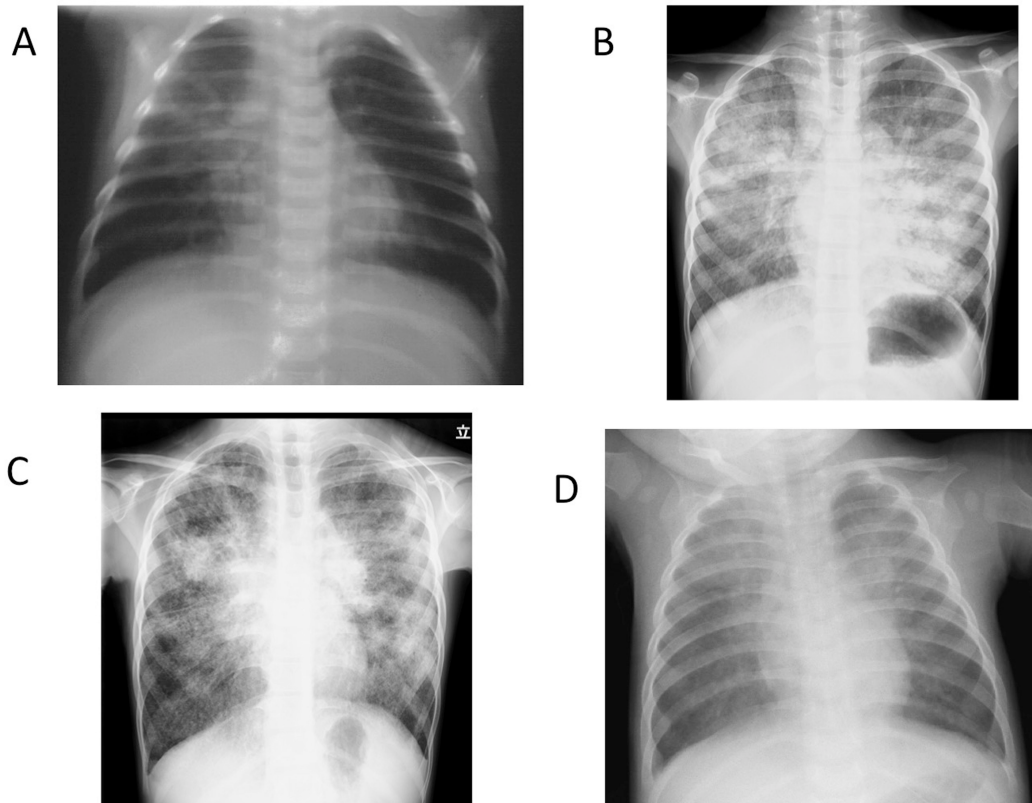
**Figure S1. Pedigrees of the three families**

A (family A): Three siblings, two boys and one girl, were affected.

B (family B): B-II-1 was affected.

C (family C): C-II-1 was affected.

All affected individuals were born to non-consanguineous parents.



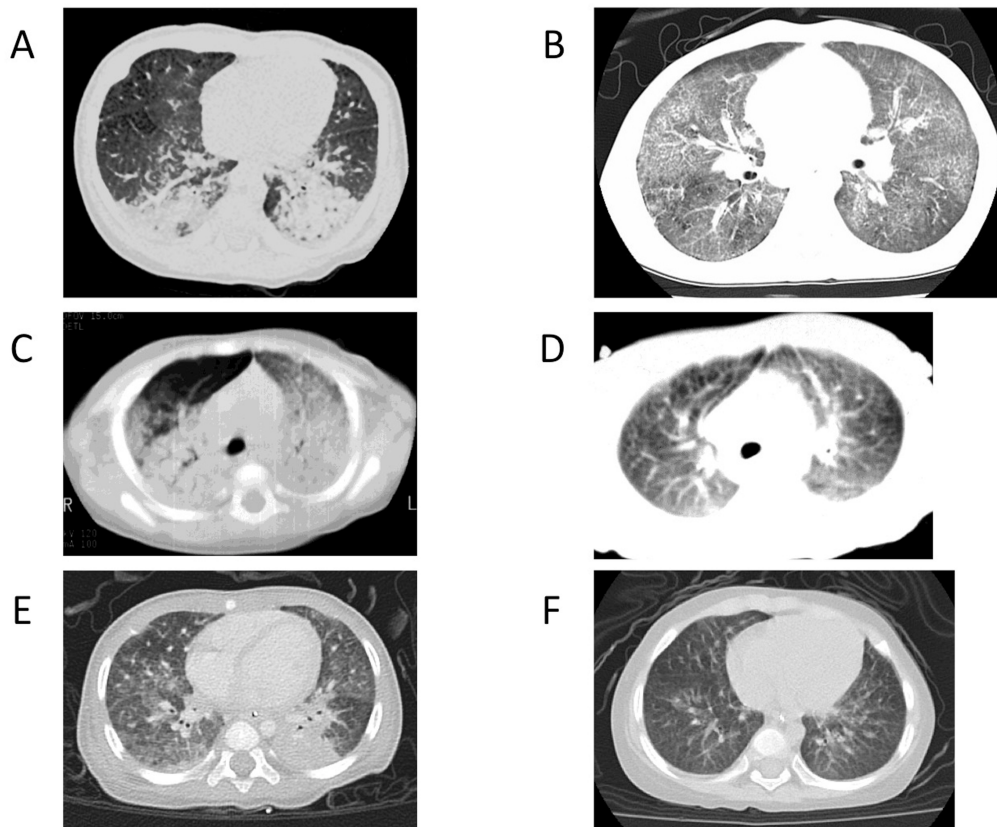
**Figure S2. Chest X-ray findings**

A (A-II-4, 42 days): Mild consolidation was first observed in the right lung.

B (A-II-4, 8 years): Her respiratory status and oxygenation function gradually deteriorated and needed home oxygen therapy at 8 years old.

C (A-II-4, 11 years): This X-ray was at the end stage of respiratory failure on noninvasive positive pressure ventilation. Diffuse infiltrations and bilateral silhouette signs were observed.

D (C-II-1, 24 days): Diffuse opacity in her lung increased gradually.



**Figure S3. Chest CT findings**

A (A-II-4, 42 days): Apparent consolidation was seen in both dependent sides.

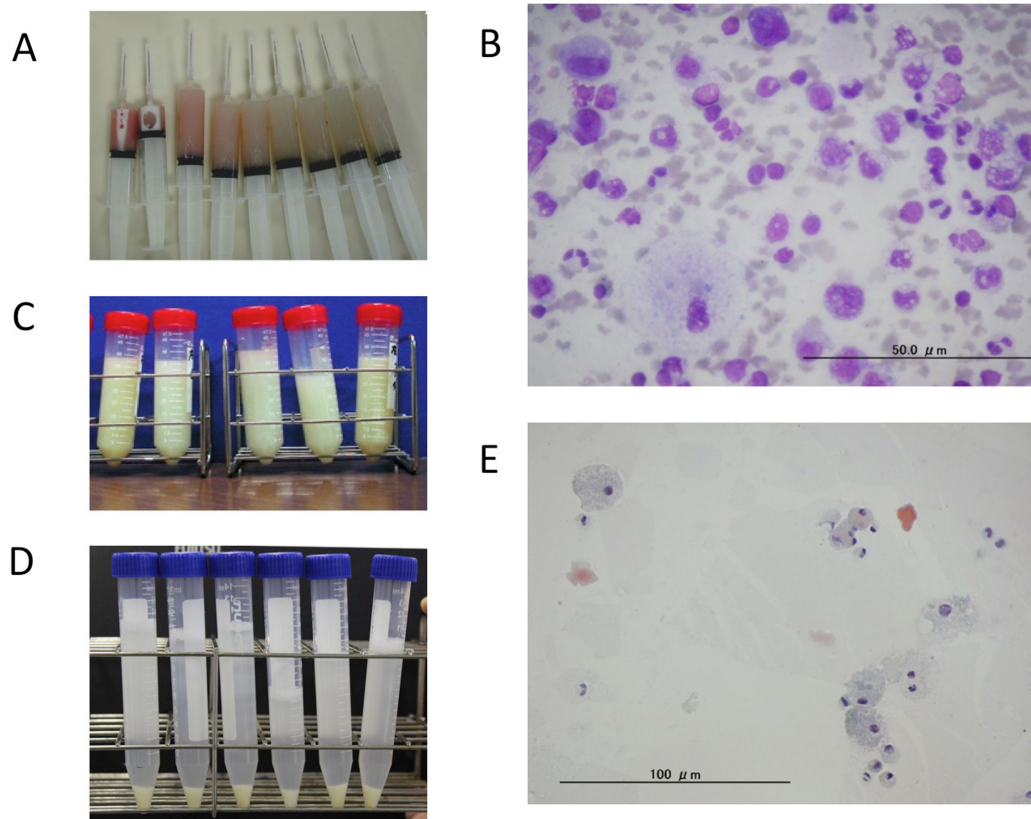
B (A-II-4, 9 years): Ground glass opacity, crazy paving pattern, and geographic opacity, findings typical of PAP, were observed.

C (B-II-1, 5 months): Severe consolidation on both dependent sides was observed.

D (B-II-1, 10 months): Consolidation on chest CT had clearly disappeared 2 months after hematopoietic stem cell transplantation (HSCT) with cord blood.

E (C-II-1, 9 months): Diffuse consolidation was observed.

F (C-II-1, 2 years): Consolidation had disappeared on follow up chest CT after HSCT.



**Figure S4. Findings in BAL fluid**

A (A-II-4, 42 days): BAL fluid obtained from the right lung at 66 days of life was milky white in appearance and contained flocculent precipitates, which stained positively with periodic acid-Schiff stain.

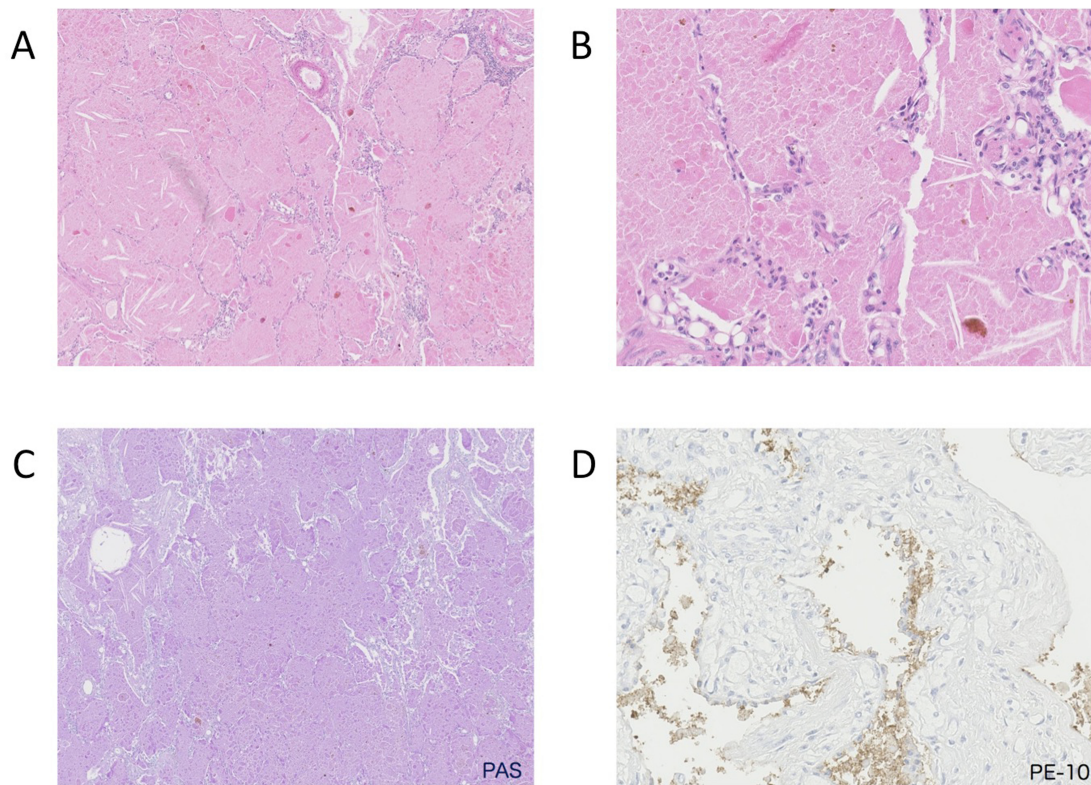
B (A-II-4, 42 days): Cytospin slide staining of BAL fluid with hematoxylin and eosin (HE). AMs were various in size and there were many CD14-positive small and non-foamy AMs.

C (B-II-1, 4 months): BAL fluid had a milky white appearance and contained abundant precipitates.

D (C-II-1, 9 months): BAL fluid obtained from the right lung at 9 months was milky

white and precipitates were observed after centrifugation.

E (C-II-1, 9 months): Cytospin staining of BAL fluid with Papanicolaou staining. AMs in BAL fluid were small and non-foamy.



**Figure S5. Histological findings of the lung of A-II-4**

A: HE staining of the lung tissues showed alveolar spaces filled with proteinaceous material, compatible with PAP (low magnification).

B: HE staining of lung tissues showed small and non-foamy AMs (high magnification).

C: The materials filling the alveolar spaces were positive for PAS.

D: Materials in alveolar spaces and AMs were not stained by PE-10 (anti-SP-A).

## SUPPLEMENTAL TABLES

**Table S1. Oligonucleotide primer sets for *OAS1***

|        | Forward              | Reverse              |
|--------|----------------------|----------------------|
| Exon 1 | AATTCAGCACTGGGATCAGG | GCACCCTGGGTTCTAGGTTT |
| Exon 2 | ATTTAGGGAGGTTTGCCTCA | CCCACCCTGCTTTAGAGAGA |
| Exon 3 | CTGGGTCTGCTGCACTTTTC | CCCTCCTCTTCCCTTCACTC |
| Exon 4 | GGATTCGTTCCAAGGAACTT | CACAGGGTTGGAGGTAGGTG |
| Exon 5 | GAGCCCTTCCTCATGTTCTG | CAAACCCCACCATTACACAA |
| Exon 6 | TCCAGATGGCATGTCACAGT | TGGCTCTGTGCCTTGAAGTT |

The coding and flanking intronic regions of three transcriptional variants of *OAS1* (RefSeq NM\_016816.3, NM\_002534.3, and NM\_001032409.2) were amplified by these primer sets.

**Table S2. Exonic sequence coverage by whole exome sequencing in family A**

| <b>ID</b>           | <b>Sample Type</b> | <b>Total (bps)</b> | <b>Mean depth</b> | <b>≥ ×5 (%)</b> | <b>≥ ×10 (%)</b> | <b>≥× 20 (%)</b> | <b>PCR duplication (%)</b> |
|---------------------|--------------------|--------------------|-------------------|-----------------|------------------|------------------|----------------------------|
| Father (A-I-1)      | WGA                | 3,683,035,312      | 110.03            | 83.1            | 76.1             | 66.6             | 13.0                       |
| Mother (A-I-2)      | WGA                | 3,849,394,706      | 115               | 83.5            | 76.9             | 67.9             | 13.3                       |
| Affected (A-II-1)   | WGA                | 3,822,951,097      | 114.21            | 90.1            | 84.8             | 76.1             | 19.5                       |
| Unaffected (A-II-2) | WGA                | 3,510,531,591      | 104.88            | 80.7            | 73.7             | 64.6             | 10.7                       |
| Affected (A-II-3)   | WGA                | 3,626,875,251      | 108.36            | 89.3            | 83.8             | 75               | 28.7                       |
| Unaffected (A-II-2) | EBV-LCL            | 4,000,057,549      | 119.5             | 96.1            | 94.8             | 91.2             | 12.8                       |
| Affected (A-II-4)   | EBV-LCL            | 3,186,587,499      | 95.2              | 95.6            | 93.8             | 88.7             | 13.3                       |

WGA, whole genome amplification; EBV-LCL, Epstein-Barr virus-transformed lymphoblastoid cell line

**Table S3. Priority scheme of homozygous variants**

| <b>Criteria for filtering</b>                                     | <b>Variant counts</b> |
|-------------------------------------------------------------------|-----------------------|
| Total variants                                                    | 4,903                 |
| Remove synonymous                                                 | 3,289                 |
| In-house exome data ( $n \leq 1/153$ )                            | 1,424                 |
| MAF $\leq 0.01$ in ESP5400                                        | 1,373                 |
| Outside Segmental duplication                                     | 1,287                 |
| Commonly shared homozygous variants among three affected children | 0                     |

**Table S4. Priority scheme of compound heterozygous variants**

| <b>Criteria for filtering</b>                                                | <b>Variant counts</b> |
|------------------------------------------------------------------------------|-----------------------|
| Total variants                                                               | 4,903                 |
| Remove synonymous                                                            | 3,289                 |
| In-house exome data ( $n \leq 1/153$ )                                       | 1,424                 |
| MAF $\leq 0.01$ in ESP5400                                                   | 1,373                 |
| Outside segmental duplication                                                | 1,287                 |
| Commonly shared heterozygous variants among three affected children          | 116                   |
| Commonly shared compound heterozygous variants among three affected children | 0                     |

**Table S5. Priority scheme of *de novo* variants**

| <b>Filtering criteria</b>                                           | <b>Variant counts</b> |
|---------------------------------------------------------------------|-----------------------|
| Total variants                                                      | 4,903                 |
| Remove synonymous                                                   | 3,289                 |
| Remove in-house exome data ( $n = 153$ )                            | 1,086                 |
| Not registered in dbSNP 135                                         | 623                   |
| Not registered in ESP5400                                           | 595                   |
| Outside Segmental duplication                                       | 570                   |
| Not observed in both parents and unaffected sibling                 | 212                   |
| Commonly shared heterozygous variants among three affected children | 1                     |

**Table S6. Read counts for *OAS1* mutation (c.227C>T) by deep sequencing**

| Called base | Father     |        | Mother     |        |
|-------------|------------|--------|------------|--------|
|             | Read count | (%)    | Read count | (%)    |
| A           | 326        | 0.10   | 328        | 0.09   |
| T           | 361        | 0.12   | 13,512     | 3.81   |
| G           | 70         | 0.02   | 54         | 0.02   |
| C           | 312,759    | 99.72  | 340,680    | 96.05  |
| N           | 117        | 0.04   | 132        | 0.04   |
| Total       | 313,633    | 100.00 | 354,706    | 100.00 |

## SUPPLEMENTAL REFERENCES

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