Severe congenital neutropenia is characterized by susceptibility to recurrent life threatening bacterial infections due to maturation arrest of neutrophils. Different studies have shown mutations in ELA 2, HAX 1, G6PC3, WAS, GF11 and VPS45 genes.1-7 In 2014, Boztug et al.8 described mutations in Jagunal homolog 1 (JAGN1) gene that play a role in neutrophils differentiation and maintenance. JAGN1 is responsible for normal ultrastructure and granulation of endoplasmic reticulum of myeloid progenitor cells. Its defect is related to increased predisposition to apoptosis. In the literature, a few cases have been reported with congenital anomalies such as cardiac and renal anomalies.

Here we report a patient with severe congenital neutropenia that showed homozygous JAGN1 mutation.

Case Report

Our patient is a 10-year-old male born to first cousin parents. He was the first child of the family and was born 1900 grams at 37 weeks of gestation. He was admitted due to neonatal sepsis for a month on the fourth day after birth. He was re-hospitalized because of ulcers and abscesses on the bilateral lower extremities which did not regress with antibiotics at 6 months of age. After these 2 episodes of hospitalizations, severe neutropenia was noticed on the complete blood count analysis.

ABSTRACT

Background. Neutrophils are essential innate cells to fight bacterial and fungal pathogens. Jagunal homolog 1 (JAGN1) mutations were recently defined as rare genetic defects causing severe congenital neutropenia. JAGN1 participates in the secretory pathway and is required for granulocyte colony-stimulating factor receptor-mediated signalling. This gene is required for normal ultrastructure and granulation of endoplasmic reticulum of myeloid progenitor cells. Its defect is related to increased predisposition to apoptosis. In the literature, a few cases have been reported with congenital anomalies such as cardiac and renal anomalies.

Case. Here we report a patient in which JAGN1 deficiency was found after several years. Apart from syndromic facial appearance we were unable to detect any other systemic malformations.

Conclusion. The causes of multisystemic features of mutations in JAGN1 gene remain unknown. JAGN1 mutations must be considered in patients with severe congenital neutropenia especially with facial dismorphism even in the absence of systemic manifestations.
In initial laboratory tests leukocyte count was 5120/mm$^3$, while absolute neutrophil count (ANC) was 100/mm$^3$, hemoglobin was 10.9 g/dl and thrombocyte count was 357000/mm$^3$. Deep neutropenia was seen in repeated complete blood counts (ANC: 0-200/mm$^3$). Bone marrow aspiration revealed maturation arrest of the neutrophils. Granulocyte – Colony Stimulating Factor (G-CSF) (5 µg/kg) treatment was started with the presumed diagnosis of Kostmann syndrome. All ANC were 0-200 / mm$^3$ without G-CSF. After G-CSF, ANCs increased up to just maximum 700 / mm$^3$. Physical examination was normal except triangular face and ears. His growth and mental development were within normal percentiles. Cardiac and abdominal investigations did not show any accompanying congenital anomalies.

He was treated for recurrent skin ulcers and abscesses until 1.5 years of age. He had recurrent infections including pneumonia, otitis media, sinusitis and skin abscess. At 3 years of age, he was hospitalized due to severe pneumonia and cavernous lesions were seen on thorax computed tomography. As anti-bacterial treatments did not cause any regression, anti-fungal and anti- tuberculosis treatments were given empirically. None of the fungal agents or M. Tuberculosis bacillus were revealed by culture. After 6 months of treatment, cavernous lesions regressed and treatment was stopped.

Serum levels of immunoglobulins and lymphocyte subtypes were within normal ranges. Because he had complaints that were suggestive of asthma and allergic conjunctivitis, skin prick test was performed and positive result was found against house dust mites. A summary of laboratory evaluation is shown in Table I.

In the investigation of genetic causes of severe congenital neutropenia, mutation analysis for ELENA, HAX1, G6PC3 and GCSF receptor mutations were found to be negative in 2012. After identification of JAGN1 deficiency in 2014, the relevant gene was sequenced and a homozygous missense mutation was detected in exon 2 of JAGN1 gene (c 130 c>T , p. His 44 Tyr) (Fig. 1).

Table I. Laboratory evaluation of the patient.

|                      | Patient’s result | Normal values          |
|----------------------|------------------|------------------------|
| Hemoglobin           | 10.9 g/dl        | 12-14 g/dl             |
| Leukocytes           | 5120 / mm$^3$    | 4000-15000 / mm$^3$    |
| Absolute neutrophil count (ANC) | 0-200 / mm$^3$ | 1500-6000 / mm$^3$    |
| Absolute lymphocyte count (ALC) | 4200 / mm$^3$ | 1500-4000 / mm$^3$    |
| Absolute eosinophil count (AEC) | 500-1800 / mm$^3$ | 0-500 / mm$^3$        |
| Absolute monocyte count (AMC) | 300-4000 / mm$^3$ | 100-1000 / mm$^3$ |
| Plateletes           | 357000 / mm$^3$  | 150000-450000 / mm$^3$ |
| IgG level            | 898 mg/dl        | 842-1943 mg/dl         |
| IgA level            | 67.2 mg/dl       | 62-390 mg/dl           |
| IgM level            | 97 mg/dl         | 54-392 mg/dl           |
| IgE level            | 17.6 KU/L        | <161.3 KU/L            |
| Lymphocyte subtypes (%) | CD3: 72,        | 55 – 78                |
|                      | CD4: 38,         | 27 – 53                |
|                      | CD8: 18,         | 19 – 34                |
|                      | CD16+56: 12,     | 4 – 26                 |
|                      | CD19: 22,        | 10 – 31                |
|                      | CD20:24,         | 10 – 30                |
|                      | HLA-DR: 18,      | 2-12                   |

Genetic analysis: JAGN1 gene (c 130 c>T , p. His 44 Tyr)
The patient is still being followed in our immunology department and receiving high doses of G-CSF (10 µg /kg). He requires hospitalization 2-3 times every year due to pneumonias and higher doses of G-CSF during infections, nevertheless, neutrophil counts have not increased adequately. Matched unrelated donor screening continues for bone marrow transplantation because of a lack of a family matched donor.

Informed consent was received from the parents before preparation of manuscript.

Discussion

In an animal study Wirnsberger et al.9, showed, that JAGN1 deficient mice do not show neutropenia, they are characterized by increased susceptibility to fungal infections due to defective killing capacity of neutrophil granulocytes. In this report, we describe a patient with JAGN 1 mutation with different features which is not defined in the original report8 and the report of Baris et al.10 The original report including 14 patients described recurrent respiratory tract infections, sepsis, skin abscess and pancolitis. Multisystemic manifestations such as short stature, convulsion, bone abnormalities (hip dysplasia, amelogenesis imperfecta, osteoporosis, scoliosis), pyloric stenosis, pancreatic insufficiency and coarctation of aorta were also reported in this cohort.8 Differently, our patient did not show any multisystem abnormalities except facial dysmorphism. Baris et al.10 described urogenital abnormalities, short stature, learning disorders, hypothyroidism and hypogammaglobulinemia. In our patient no immunologic abnormalities were seen besides neutropenia. Now his main symptoms include cough, rhinorrhea and conjunctivitis, after recovering from serious infections which were frequent during his younger childhood years. They were considered due to a house dust mite allergy. An allergic condition has not been reported before in these patients. We think that it may be seen co-incidentally and needs further investigation.

Like the other patients in the original cohort, our patient did not respond to high dose G-CSF treatment.8 Interestingly, in the mice study, the authors stated that JAGN1 knock out mice’s neutrophils showed increased killing capacity with GM-CSF.9 We could not try this type of CSF in our patient during his infections.

Homozygous missense mutation in the exon 2 detected in our patient was the same as the Turkish patients in the study of Baris et al.10 and the original cohort.8 In the series containing 14 patients, there were 2 Turkish patients carrying the same mutation as our patient. Skull bone thickness due to extramedullary hematopoesis were reported as a different clinical finding. We think that this mutation may be a common mutation in the Turkish population and may be used to screen for the etiology of severe congenital neutropenia in Turkish patients. Because of lack of hypogammaglobulinemia and multisystemic manifestations in our patient as in previous reported Turkish patients, we think that this type of mutation does not cause the same phenotype in all patients. The cause of multisystemic features of mutations in JAGN 1 gene remains unknown. All cases presented previously are summarized in Table II.

In conclusion, 3 patients were reported with the same JAGN 1 mutations and all were Turkish worldwide, all of them manifested with multisystemic congenital anomalies and neutropenia. We suggest that JAGN 1 gene mutation must be considered in patients with...
| Gender | Country  | Mutation          | Beginning symptoms                                      | Extrahematopoetic manifestations | Treatment and Clinical status |
|--------|----------|-------------------|--------------------------------------------------------|----------------------------------|------------------------------|
| F      | Algeria  | c.3G>A; p.Met1Ile | ENT infections, aphthosis, perianal cellulitis, skin abscesses | None                             | Alive without treatment      |
| F      | Algeria  | c.3G>A; p.Met1Ile | ENT infections,                        | Short stature (height of 1.46 m) | Alive without treatment      |
| M      | Algeria  | c.3G>A; p.Met1Ile | Aphthosis, skin abscesses, balanitis, pneumonitis, lung abscess, osteitis perianal cellulitis | Pyloric stenosis                       | Alive without treatment      |
| F      | Algeria  | c.3G>A; p.Met1Ile | Otitis, paraodontopathy                  | Scoliosis, dental malformations   | Alive without treatment      |
| M      | Algeria  | c.3G>A; p.Met1Ile | ENT infections, aphthosis, skin abscesses, pneumonitis, lung abscess, perianal cellulitis | None                             | Alive without treatment      |
| F      | Iran     | c.59G>A; p.Arg20Glu| Upper respiratory tract infections, pneumonia, skin abscesses | Febrile convulsion, focal epilepsy | Alive without treatment      |
| M      | Turkey   | c.130C>T; p.His44Tyr | Upper respiratory tract infections, pneumonia, skin and perianal abscesses, sepsis (Haemophilus influenza) | Extramedullary hematopoiesis with thickening of skull bones | Alive without treatment      |
| F      | Turkey   | c.130C>T; p.His44Tyr | Upper respiratory tract infections, skin abscess | Bilateral hip dysplasia, extramedullary hematopoiesis with thickening of skull bones | Alive without treatment      |
| F      | Iran     | c.40G>A; p.Gly14Ser | Skin abscesses, onycholysis               | None                             | Alive without treatment      |
| M      | Israel   | c.297C>G; p.Tyr99  | Aspergillosis (none after HSCT)           | Severe osteoporosis and repeated bone fractures (continuing after HSCT) | Alive with HSCT              |
| F      | Morocco  | c.485A>G; p.Gln162Arg | Skin abscesses, omphalitis, pancolitis | Lipomatosis, pancreatic insufficiency, bone abnormalities, dental malformations | Died at age 5 years owing to pancolitis and septicemia |
| F      | Albenia  | c.63G>T; p.Glu21Asp | Upper respiratory tract infections, pneumonia, skin abscess | Short stature (5 cm below third percentile), amelogenesis imperfecta, neurodevelopmental delay | Alive without treatment      |
| Gender | Country | Mutation | Beginning symptoms | Extrahematopoetic manifestations | Treatment and Clinical status |
|--------|---------|----------|--------------------|---------------------------------|-------------------------------|
| Patient 13 | F | Pakistan | c.485>A>G; p.Gln162Arg | ENT infections, upper respiratory tract infections, pneumonia, sepsis (Escherichia coli) | Failure to thrive (height 5 cm below third percentile, weight 3.8 kg below third percentile), coarctation of the aorta, mild developmental delay | Alive, awaiting HSCT |
| Patient 14 | F | Germany | c.35_43del CCGACGGCA; p.Thr12_Gly14del | Pneumonia (none after HSCT), bronchiectasis | None | Alive with HSCT |
| Patient 15 | M | Turkey | c.130>C>T, p. His44Tyr | Gluteal abscess, cervical lymphadenopathies, pneumonia, bronchiectasis, diarrhea, otitis and gingivitis | Failure to thrive, dysmorphic face, hypothyroidism, hypospadias and left undescended testis, hypogammaglobulinemia | Alive without treatment |
| Patient 16 | F | Turkey | c.130>C>T, p. His44Tyr | Recurrent skin abscesses, otitis and pneumonia | Learning disability, for triangular face, amelogenesis imperfecta, gingival hypertrophy and short stature, hypogammaglobulinemia | Alive without treatment |
| Our Patient | M | Turkey | c.130>C>T, p. His44Tyr | Neonatal sepsis, ulcers and abscesses on lower extremities, recurrent pneumonia, otitis media and sinuitis | Triangular face, extrovert ears, allergic rhinoconjuntivitis with sensitization against house dust mites | Alive without treatment |

ENT: Ear nose throat, HSCT: Hemopoetic stem cell transplantation.
severe congenital neutropenia especially those with facial dysmorphism even in the absence of multisystemic manifestations like our patient.

Acknowledgement
All Turkish authors are primary clinicians of the patient and wrote the manuscript. The authors from Austria performed genetic analysis of the patient. We thank all contributors and the patient’s family as well as the patient.

REFERENCES
1. Klein C. Genetic defects in severe congenital neutropenia: emerging insights into life and death of human neutrophil granulocytes. Annu Rev Immunol 2011; 29: 399-413.
2. Grenda DS, Murakami M, Ghatax J, et al. Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. Blood 2007; 110: 4179-4187.
3. Klein C, Grudzien M, Appaswamy G, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). Nat Genet 2007; 39: 86-92.
4. Boztug K, Appaswamy G, Ashikov A, et al. A novel syndrome with congenital neutropenia caused by mutations in G6PC3. N Engl J Med 2009; 360: 32-43.
5. Devriendt K, Kim AS, Mathijs G, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat Genet 2007; 27: 313-317.
6. Person RE, Li FQ, Duan Z, et al. Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2. Nat Genet 2003; 34: 308-312.
7. Stepensky P, Saada A, Cowan M, et al. The Thr224Asn mutation in the VPS45 gene is associated with congenital neutropenia and primary myelofibrosis of infancy. Blood 2013; 121: 5078-5087.
8. Boztug K, Järvinen PM, Salzer E, et al. JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia. Nat Genet 2014; 46: 1021-1027.
9. Wirnsberger G, Zwolanek F, Stadlmann J, et al. Jagunal homolog 1 is a critical regular of neutrophil function in fungal host defense. Nat Genet 2014; 46: 1028-1033.
10. Baris S, Karakoc-Aydıner E, Ozen A, et al. JAGN1 deficient severe congenital neutropenia: two cases from the same family. J Clin Immunol 2015; 35: 339-343.