Extending the Phenotype and Identification of a Novel Candidate Gene for Immunodeficiency in 5q11 Microdeletion Syndrome

Veronica Arora^a Shruti Aggarwal^a Sunita Bijarnia^a Meena Lall^a Anju Joshi^a
Ratna Dua-Puri^a Umang Arora^b Ishwar Verma^a

^aInstitute of Medical Genetics and Genomics, Sir Ganga Ram Hospital, and ^bAll India Institute of Medical Sciences, New Delhi, India

Established Facts

- To date, only 13 cases of 5q11.2 microdeletion are reported. However, the phenotype is not precisely delineated. Features described so far are intellectual disability, dysmorphism, nonspecific ocular anomalies, and heart defects.
- *DHX29* is the causative gene for developmental delay, dysmorphism, and heart defects. It encodes for an ATP-dependent RNA helicase, which is involved in initiation of translation.

Novel Insights

- The present case extends the phenotypic spectrum of 5q11.2 microdeletion with immunodeficiency, skeletal asymmetry, joint laxity, hypoplastic nares, and agenesis of corpus callosum.
- A common critical region spanning 1.4 Mb (54–55.4) and involving 14 genes has been defined for all the published cases.
- We propose that haploinsufficiency of *IL6ST* accounts for the immunodeficiency observed in these patients, as it plays a critical role in both innate and adaptive immunity.

Keywords

Common critical region · *IL6ST* · Immunodeficiency · 5q11 microdeletion syndrome

Abstract

Array CGH has led to the delineation of innumerable microdeletion syndromes. We present a patient with a 7-Mb deletion at 5q11.2 with previously unreported features, such as immunodeficiency, asymmetry of hands and feet, joint laxity, and agenesis of corpus callosum. The clinical features of this patient are compared with 13 patients reported previ-

ously. A common critical region (CCR) of 1.4 Mb (54–55.4 Mb) is defined in all cases including the present one. Of the 14 genes present in CCR, *IL6ST* is proposed to be the candidate gene for immunodeficiency observed in some of these patients. *IL6ST* encodes gp130, a signal transduction protein for various interleukins and cytokines. It is involved in the generation of both T and B lymphocytes as well as the production of acute-phase reactants. Microdeletion 5q11.2 should be considered as a recognisable syndrome based on the common phenotype and the novel features described.

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Whole-genome microarray has revolutionised the field of cytogenetics, leading to the delineation of innumerable microdeletion syndromes [Slavotinek, 2008]. In cases with developmental delay and/or dysmorphism, it has replaced conventional FISH and karyotype studies and is now considered the first-line investigation [Miller et al., 2010]. Prescott et al. [2005] first described 5q11.2 microdeletion syndrome in a patient with short stature, learning difficulties, tetralogy of Fallot, bifid uvula, and velopharyngeal defects. Subsequently, it was reported in 12 other patients with dysmorphism, varying degrees of intellectual disability, and nonspecific ocular defects (hypermetropia, esotropia, and myopia). In 2 of these patients, the phenotype resembled CHARGE syndrome. Haploinsufficiency of *DHX29* is implicated as the culprit gene for the phenotypic characteristics of this syndrome. It encodes an ATP-dependent RNA helicase that is involved in the initiation of RNA translation and cell proliferation. [Snijders Blok et al., 2014]. Later, 2 more genes (*SLC38A9* and *GP1B1*) were linked to the genesis of this phenotype [Fontana et al., 2016], of which *SLC38A9* is present in the common critical region (CCR) defined in the present study.

Herein, we describe a 15-year-old girl with dysmorphic features, immunodeficiency, and learning difficulties, who was found to harbour a 7.5-Mb deletion at chromosome 5q11.2. This report also compares the clinical and molecular features of previously published cases. CCR for all the cases is defined, and *IL6ST* is proposed as the candidate gene for immunodeficiency.

Case Report

A 15-year-old girl, the second born to nonconsanguineous parents, was referred for evaluation of facial dysmorphism, poor scholastic performance, and recurrent infections. She was delivered at 38 weeks of gestation by caesarean section, carried out in view of foetal distress. Antenatal and perinatal periods were uneventful. She cried immediately after birth, and the APGAR score at 5 min was 9. The anthropometry at birth was appropriate for age. Her weight was 2,885 g (15–50th centile), length 51 cm (50–85th centile), and head circumference 34 cm (15–50th centile) as per WHO growth charts. Dysmorphism and developmental delay were first noted at 2 years of age. All developmental milestones were found to be delayed: she achieved sitting independently at 14 months, walking at 23 months, and spoke her first sentences at 33 months of age; she was reported to be a slow learner at school. There is a history of recurrent respiratory tract and ear infections since infancy. She suffered from 4 episodes of lower respiratory tract infections in the past year, 3 and 5 episodes in 2016 and 2015, respectively. Strabismus was noted in the left eye at the age of 3 years. On evaluation, she was found to have hypermetropia and needed

glasses. At present, she is in the 5th grade in school, interacts well with peers but has a below average scholastic performance.

On examination, she was alert and interactive. She was noted to have a long face, sloping forehead, strabismus, hypertelorism, hirsutism, underdeveloped ala nasi, prominent cheeks and philtrum, micrognathia, and a downturned mouth (Fig. 1A–C). Asymmetry was noted in the hands and feet, the right side being longer than the left by 2.5 cm (Fig. 1D, E). Hyperlaxity of the joints was present. Evaluation of higher mental functions revealed poor sustained attention and long-term memory as well as the inability to perform simple calculations. A convergent squint was observed, but there was no nystagmus or oculomotor apraxia. Her head circumference was 51 cm (50th centile), height 160.5 cm (50–75th centile), weight 35 kg (<3rd centile), and arm span 162 cm, as per WHO charts. All other systemic examination parameters were within normal limits.

Methods and Results

A haemogram revealed haemoglobin to be 13.2 g/dL (11.5–15 g/dL), packed cell volume 39.8%, RBC count 4.33 million/mm³, MCH 30.5 pg/dL, MCHC 33.2 g/dL, RDW 12.3%, and platelet count 100,000/mm³. The total leucocyte count was 3,900 cells/μL (normal range 4,000–11,000). The differential leucocyte count showed decreased lymphocytes with eosinophilia (neutrophils 68%, lymphocytes 16%, monocytes 6%, eosinophils 9%, and basophils 1%). In view of recurrent infections, her immunological profile showed low IgG titres of 121 mg/dL (716–1,711 mg/dL), low IgA 25 mg/dL (47–249 mg/dL), normal IgM 45 mg/dL (23–259 mg/dL), high IgE 920 IU/mL (150–300 IU/mL), CD4+ T cells 44% (27–53%), CD8+ T cells 18% (14–44%), CD19+ B cells 3% (8–27%), and CD16+CD56+ NK cells 0.5% (2–27%). The immune profile was performed at 3 different times and showed a similar pattern (Table 1). A sputum culture done for recurrent lower respiratory tract infections showed gram positive diplococci, *Streptococcus pneumoniae* (on 3 occasions) and *Haemophilus influenzae* (on 2 occasions).

The girl was tested in view of developmental delay. TSH 3.2 mIU/mL (0.3–5 mIU/mL), free T4 4.3 ng/dL (4–12 ng/dL), dried blood spot acylcarnitines, and urine organic acid analysis were within the normal range. A formal assessment revealed an IQ of 53, indicating mild intellectual disability.

Radiographs of the hands and feet were consistent with the asymmetry observed on clinical examination. The right side was longer than the left side by 2.5 cm in both the upper and lower limbs. Brain MRI showed cerebral atrophy and partial agenesis of the corpus callosum

Fig. 1. **A–C** Clinical photographs of the patient showing tall and slender build, long face, hirsutism, hypertelorism, strabismus, sloping forehead, underdeveloped ala nasi, long prominent philtrum, micrognathia, and a downturned mouth. **D, E** Asymmetry of

the hands and feet. Note the right side is longer than the left. **F** MRI image: sagittal plane, cerebral atrophy and absent splenium of corpus callosum (arrow) are shown.

(Fig. 1F). Echocardiography and ultrasonography of the abdomen did not reveal any abnormalities.

In view of dysmorphism and developmental delay, a chromosomal aetiology was considered. The karyotype of the proband was 46,XX,del(5)(q11.2), consistent with a deletion in 5q11.2 (Fig. 2). Whole-genome microarray-based hybridisation was performed on an AGILENT 4x180K (CGH+SNP) array slide and evaluation was done using cytogenomics software version 3.0.6.6 based on UCSC build 37 (hg19). It revealed a clinically significant 7.5-Mb deletion at 5q11.2q12.1 starting from 54,097,755 to 61,138,711 bp in chromosome 5 (Fig. 3). No other copy number variations or regions of loss of heterozygosity were observed. The karyotypes of the father and the mother were normal, and there were no balanced rearrangements. Array-CGH study in the parents did not reveal any copy number changes, thus proving the de novo nature of the deletion.

Discussion

To date, only 13 cases of the microdeletion 5q11.2 have been reported (Table 2). We present a 15-year-old girl with a de novo 7.5-Mb deletion involving a large part of the 5q11.2 region. Although a distinct phenotype is

Table 1. Immune markers done in 4 consecutive years

	January 2015	August 2016	May 2017	February 2018
TLC (cells/ μ L)	4,100	3,750	3,900	4,050
Differential leucocyte count				
N	62	67	68	60
L	15	13	16	14
M	8	5	6	10
E	13	10	9	14
B	2	0	1	2
Immunoglobulins				
IgG (range 716 – 1,711 mg/dL)	96	108	121	77
IgA (range 47 – 249 mg/dL)	14	17	25	11
IgM (range 23 – 259 mg/dL)	28	23	45	21
IgE (range 150 – 300 IU/mL)	442	1,020	920	904
CD4+ T (range 27 – 53%)	22	18	44	22
CD19+ (range 6 – 27%)	2	1.5	3	2.8
NK cells (range 2 – 27%)	0.2	0.8	0.5	0.9

TLC, total leucocyte count.

not defined for this microdeletion syndrome, published cases share many common clinical characteristics (Table 2). The proband has some overlapping features with the previously reported cases, including intellectual disability, ocular defects, sloping forehead, low-set posterior

Fig. 2. Chromosome 5 of the proband showing the 5q11.2 deletion (left), and the normal chromosomes of the mother (middle) and the father (right).

Fig. 3. Microarray image showing the 7.5-Mb deletion together with the genes in this region.

Fig. 4. Chromosome 5q11 deletion for all the reported patients as well as the present case. The common critical region is highlighted (54–55.4 Mb), and the genes located in this region are shown below.

only rotated ears, micrognathia, and arachnodactyly. In addition, asymmetry in hands and feet, prominent maxillae, hypoplastic nares, and joint laxity were observed. Brain MRI showed the presence of partial agenesis of corpus callosum and cortical atrophy, which have not been previously reported in this microdeletion syndrome.

CCR for all the previous cases spans from 54,097,755 to 55,412,230 bp and contains 14 OMIM genes (Fig. 4). *DHX29* encodes a DEXH-box protein that is required for the translation of mRNAs with structured 5' UTRs. It is important for initiation of translation and cell proliferation. Silencing of this gene has been implicated as the main contributor to the dysmorphisms, as well as growth

Table 2. Comparison of clinical, immunological and radiological features of all reported patients

Reference	Lindstrand et al., 2014			Snijders-Blok et al., 2014			Prescott et al., 2005	Fontana et al., 2016	De Jong et al., 2010	ECARUCA database	Jaillard et al., 2011		Decipher ID-291360	Present case	Summary statistics ^a	
	P1	P2	P3	P4	P5	P6					P7	P8				P9
Patients	Coordinates on chromosome 5q (Mb)															
	Deletion size (Mb)															
	Age at presentation, years															
	Gender															
	Developmental delay ^b															
	Intellectual disability ^b															
	Behaviour															
	Recurrent infections															
	Seizures															
	Deafness															
	Build															
	Normal, slender															
	Normal, thin															
	Short															
	Short stature = 4/13 (31)															
Tall, slender																
 																
Short stature =																

^a Values in parentheses indicate percent values. ^b +, mild; ++, moderate; +++, severe. ICS, intercostal space; NA, not available; PDA, patent ductus arteriosus; TOF, tetralogy of Fallot; VPD, velopharyngeal defects; ys, years. Novel features are listed under Facial dysmorphisms and Clinical features.

deficiencies, seen in some of these patients [Snijders Blok et al., 2014].

The proband had symptoms consistent with immunodeficiency. Testing revealed a deficiency of both humoral and cellular immunity, which included reduced levels of immunoglobulins (IgG and IgA), T cells, and markedly decreased B and NK cells. There was mild eosinophilia. The deleted region in chromosome 5q11 contains 45 genes, amongst which *IL6ST* is identified as the probable gene related to immunodeficiency due to its established role in immune responses. The protein encoded by this gene is a signal transducer shared by many cytokines, including interleukin 6 (IL-6), ciliary neurotrophic factor, leukaemia inhibitory factor, and oncostatin M. This protein functions as a part of the cytokine receptor complex [Tanaka et al., 2014; Schwerd et al., 2017]. It has also been called the B-cell stimulatory factor 2 (BSF-2), based on the ability to induce differentiation of activated B cells into antibody-producing cells, especially IgG producing IL-21 [Hirano et al., 1986; Ma et al., 2012]. Furthermore, IL6 has a prominent role in synthesis and secretion of acute-phase reactants, which have multiple and complex roles in integrated host defences against bacterial pathogens such as *Klebsiella pneumoniae* and *Streptococcus pneumoniae* [Quinton et al., 2009]. Thus, the recurrent respiratory tract infections in this patient can be explained by a poor humoral immune response.

Defective IL6 signalling due to haploinsufficiency of *IL6ST* could play an important role in the pathogenesis of the immunodeficient state. In addition, recurrent infections have been previously described in 4 patients with 5q11.2 microdeletion syndrome, and all 4 were haploinsufficient for this gene (55.23–55.29 Mb), thus substantiating this hypothesis. Furthermore, Schwerd et al. [2017] presented a patient with recurrent infections, eczema, bronchiectasis, high IgE, eosinophilia, defective B cell

memory, and an impaired acute-phase response. The patient was found to have a homozygous loss-of-function mutation of *IL6ST* (encoding gp130 p.N404Y). The present study confirms the disease-causing role of this gene. The more severe phenotype in the patient described by Schwerd et al. [2017] compared to that in the present study is probably due to the bi-allelic mutation in their case, which resulted in complete abolition of protein production and function.

In summary, we report a patient with a 5q11.2 microdeletion and novel features of immunodeficiency, asymmetry of hands and feet, and agenesis of corpus callosum, thus extending the phenotypic spectrum of this rare microdeletion syndrome. Though the microdeletion is larger than CCR, these features have not been previously reported including the patients with deletions beyond the 5q11 region. Microdeletion 5q11.2 should be considered a recognisable microdeletion syndrome, based on the previously described and the newly added features, with 54–55.4 Mb being the CCR. We also emphasise the need for neurological, immune, and skeletal system examination in these patients. *IL6ST* is proposed to be the candidate gene for immunodeficiency due to its critical role in immune regulation. However, further characterisation of immune functions in additional patients with 5q11 microdeletion is required to validate this hypothesis.

Statement of Ethics

This study was approved by the ethics committee, and written informed consent was obtained.

Disclosure Statement

All the authors declare no conflicts of interest.

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