Lymphstasis in a Boy with Noonan Syndrome: Implication for the Development of Skeletal Features

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Abstract. We report on a Japanese boy with Noonan syndrome who had short stature, bilateral cryptorchidism, poor pubertal development, mild mental retardation, complex cardiac lesions consisting of hypertrophic cardiomyopathy, mitral valve stenosis and insufficiency, subvalvular aortic stenosis, and single coronary artery, and various dysmorphic features including hypertelorism, epicanthic folds, low set malrotated ears, high arched palate, micrognathia, webbed neck, low posterior hairline, shield chest, pectus excavatum, cubitus valgus, borderline short metatarsals, lymphedema, redundant skin, and nail dysplasia. Because of marked lymphedema in the bilateral lower legs, lymphatic scintigraphy was carried out at 13.3 years of age, indicating extreme lymphstasis in the lower extremities, severe lymphstasis in the forearm, the elbow, and the axillary regions, moderate lymphstasis around the ascending aorta, and mild lymphstasis in the bilateral lungs. The results, in conjunction with those suggested in Turner syndrome, imply that lymphatic hypoplasia/dysplasia and resultant distended lymphatics and lymphedema are relevant to the development of not only soft tissue and visceral anomalies but also skeletal anomalies in Noonan syndrome.

Key words: Noonan syndrome, Skeletal features, Lymphstasis, Lymphatic dysplasia/hypoplasia, Deformation
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NOONAN syndrome is an autosomal dominant disorder characterized by short stature, delayed puberty, right-sided cardiac anomalies, and multiple characteristic dysmorphic features [1, 2]. Mild mental retardation, hearing difficulty, and bleeding diathesis are also occasionally observed in affected individuals, as are hypoplastic external genitalia and cryptorchidism in affected males.

Recently, it has been shown that heterozygous missense mutations of the gene for *PTPN11* (protein-tyrosine phosphatase, nonreceptor-type 11) on 12q24 cause Noonan syndrome, probably because of gain-of-

function effects leading to excessive *PTPN11* activity [3–5]. Since *PTPN11* is widely expressed and is involved in multiple signal transduction pathways in cooperation with a variety of molecules including growth factors, cytokines, and hormones [6], this would explain the wide range of clinical features in Noonan syndrome. However, it is probable that a gene(s) other than *PTPN11* is also related to the development of Noonan syndrome, because *PTPN11* mutations remain undetected in nearly half of the patients with typical Noonan syndrome [3–5].

For the pathogenesis of Noonan syndrome, lymphatic hypoplasia/dysplasia has been suggested to play an important role in the development of clinical features [7], although it remains to be clarified how mutations of *PTPN11* and other gene(s) for Noonan syndrome lead to lymphatic hypoplasia/dysplasia. Lymphatic hypoplasia/dysplasia should cause lymph fluid stasis, leading to lymphatic distension and lymphedema [8].

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Thus, soft tissue stigmata such as webbed neck can be regarded as the deformational consequences resulting from distended nuchal and peripheral lymphatics and lymphedema, and right-sided cardiac anomalies such as pulmonary valve stenosis can be explained by the compressive effect of pulmonary lymphangiectasis on cardiovascular system [7, 8]. It is uncertain, however, whether skeletal features in Noonan syndrome are also ascribed to lymphatic hypoplasia/dysplasia.

Here, we report on lymphatic scintigrams in a boy with Noonan syndrome, and discuss on a potential relationship between lymphatic hypoplasia/dysplasia and skeletal features.

Case Report

This Japanese boy was born to non-consanguineous parents at 41 weeks of gestation after an uncomplicated pregnancy. At birth, his length was 50.5 cm (+0.3 SD) and his weight 3.36 kg (+0.4 SD). At 1.5 years of age, he was referred to us because of failure to thrive and cardiac murmur. Extensive studies were carried out including chest radiography, electrocardiography, echocardiography, and cardiac catheterization, indicating hypertrophic cardiomyopathy, mitral valve stenosis and insufficiency, subvalvular aortic stenosis, and single coronary artery. He was treated with digitalis and diuretics thereafter, and received a surgical operation to replace mitral valve with a prosthetic one and to resect subaortic tissue at 7.3 years of age. At 16.2 years of age, he had bronchopneumonia, and died of cardiac failure with atrial fibrillation.

Physical examination was repeated throughout his clinical course, showing characteristic Noonan features. They included: (1) short stature (<-2 SD), which did not respond to GH therapy from 11.2 to 13.9 years of age; (2) bilateral cryptorchidism, which was repaired by orchidopexy at 13.2 years of age; (3) primary hypogonadism and resultant poor pubertal development, which required testosterone therapy from 15.5 years of age; (4) mild mental retardation; and (5) various dysmorphic features including hypertelorism, epicanthic folds, low set malrotated ears, high arched palate, micrognathia, webbed neck, low posterior hairline, shield chest, pectus excavatum, cubitus valgus, borderline short 2nd and 3rd metatarsals, generalized lymphedema, redundant skin in the upper and lower extremities, and nail dysplasia of all the fingers and

Fig. 1. Photograph of the boy with Noonan syndrome at 13.3 years of age.

toes (Fig. 1). In particular, lymphedema in the lower legs became severe from about 12 years of age and affected his daily life despite management with an elastic bandage.

Cytogenetic and molecular studies

After obtaining informed consent, chromosome analysis was performed for 50 peripheral lymphocytes by G-banding. Mutational analysis was performed for all the 15 exons and their flanking introns of *PTPN11* by the previously described method [5], using leukocyte genomic DNA. Mutational analysis was also carried out for *SHOX* (short stature homeobox containing gene), which is located on the short arm pseudoautosomal region of the human sex chromosomes and is responsible for growth failure and Turner skeletal features [9, 10], by fluorescence *in situ* hybridization for lymphocyte metaphase spreads and by direct sequencing of the entire coding region for leukocyte genomic DNA, as previously reported [11, 12].

The karyotype was 46, XY with no demonstrable mosaicism. No mutation was identified for *PTPN11*, nor was submicroscopic deletion or intragenic mutation found for *SHOX*.

Lymphatic scintigraphy

Because of marked lymphedema in the bilateral lower legs, lymphatic scintigraphy was carried out after thorough consultation at 13.3 years of age. In brief, 0.5 ml of solution containing 99m Technetium-labeled

phytic acid was subcutaneously injected from the distal part of the bilateral dorsal pedis, and scintigrams were obtained at 1, 3, and 6 hours after the injection. One week later, the radiolabeled material was similarly injected from the 2nd and the 3rd interdigital areas of the right hand, and scintigrams were obtained at the same time interval. For controls, lymphatic scintigrams in an adult female with breast cancer and a 17 2/12-year-old girl with 45, X Turner syndrome were utilized. The adult female received lymphatic scintigraphy for the assessment of metastasis, and the Turner girl underwent lymphatic scintigraphy for the estimation of extreme lymphedema in the bilateral lower extremities. Clinical features of the Turner girl included short stature (-3.0 SD), lack of pubertal development, epicanthic folds, low set malrotated ears, high arched palate, micrognathia, mild puffy hands, nail dysplasia, and pigmented nevi, in addition to the severe lymphedema in the lower extremities. Cubitus valgus was at

the borderline level, and cardiac lesion was absent.

Examination of lymphatic flow from the lower extremities showed marked lymphstasis in the whole lower extremities and failed to detect the thoracic duct. The lymphstasis was similarly delineated at 1, 3, and 6 hours after the injection. Investigation of lymphatic flow from the right upper extremity revealed marked lymphstasis in the forearm, the elbow, and the axillary regions (Fig. 2). Furthermore, moderate lymph fluid retention was indicated around the ascending aorta, and mild lymphstasis was detected in the bilateral lungs, in addition to strong signals in the liver. In the adult females, lymphatic imaging was delineated in the healthy regions shortly after the injection, and almost disappeared at 1 hour after the injection. In the Turner girl, lymphstasis was severe in the bilateral lower limbs and mild in the upper limbs with no obvious lymph fluid retention in the elbow regions, around the aorta, or in the lungs.

Fig. 2. Lymphatic scintigram of this boy at 13.3 years of age, indicating severe lymphstasis in the forearm, the elbow (thick arrow), and the axillary regions, moderate lymphstasis around the ascending aorta (thin arrow), and mild lymphstasis in the bilateral lungs. The liver is also delineated.

Discussion

This boy had various clinical features compatible with Noonan syndrome. Since *PTPN11* mutation was undetected, it is likely that a hitherto unknown gene for Noonan syndrome has been mutated in this boy. In this regard, it has been reported that clinical features are comparable between *PTPN11* mutation positive and negative patients with Noonan syndrome, except for higher prevalence of pulmonary stenosis in mutation positive patients and that of hypertrophic cardiomyopathy in mutation negative patients [4]. Although this boy had left-sided complex cardiac anomalies, such cardiac lesions have occasionally been reported in Noonan syndrome [13].

Lymphatic scintigraphy of this boy revealed extreme lymphstasis in the lower extremities, severe lymphstasis in the forearm, the elbow, and the axillary regions, moderate lymphstasis around the ascending aorta, and mild lymphstasis in the bilateral lungs. This provides further support for the presence of lymphatic hypoplasia/dysplasia in Noonan syndrome, because lymphstasis is ascribed to lymphatic hypoplasia/dysplasia [8]. Furthermore, since lymphstasis and resultant lymphatic distension and lymphedema should exert a mechanical force on the adjacent tissues and organs, as has been suggested in Turner syndrome [14], this would explain the development of most, if not all, soft tissue and cardiovascular anomalies of this boy.

For the relationship between lymphatic hypoplasia/dysplasia and soft tissue and visceral stigmata, the comparison between Turner and Noonan syndrome appears to be noteworthy. It has been reported that peripheral lymphstasis is shared in common by the two syndromes, whereas para-aortic lymphstasis is frequent in Turner syndrome [15] and pulmonary lymphstasis is prevalent in Noonan syndrome [7]. This would explain why peripheral soft tissue stigmata are common to Turner and Noonan syndrome, whereas cardiovascular anomalies are primarily left-sided in Turner syndrome [14] and right-sided in Noonan syndrome [1, 2]. Although the mechanism leading to the difference in the distribution of lymphstasis remains unknown at present, this would be clarified by molecular cloning and characterization of the putative Turner-related lymphogenic gene shared by Xp11.4 and Yp11.3 [10, 17], and by the elucidation of the molecular network of Noonan-causative genes including *PTPN11* [3].

In considering the underlying factor(s) for Noonan skeletal features, it would be useful to summarize the current knowledge about the pathogenesis of Turner skeletal features, because most skeletal features are common to the two syndromes. In this regard, it has been shown that *SHOX* is almost exclusively expressed in faciocervical and distal limb regions where Turner skeletal features are recognized postnatally [17], and that *SHOX* escapes X-inactivation and exerts the dosage effect in patients with sex chromosome aberrations [18]. Furthermore, clinical studies in patients with *SHOX* haploinsufficiency caused by intragenic mutations, submicroscopic deletions, and cytogenetically recognizable Xp deletions have suggested the following: (1) *SHOX* haploinsufficiency results in not only short stature but also various Turner skeletal features; (2) rare Turner skeletal features in the distal limb region such as Madelung deformity and mesomelia are primarily facilitated by the bone maturing effect of gonadal estrogens; (3) common Turner skeletal features in the craniofacial and cervical region such as high arched palate, micrognathia, and short neck are largely contributed by the bone compressive effect of distended lymphatics and lymphedema; and (4) common Turner skeletal features in the distal limb region such as cubitus valgus and short metacarpals are subject to both the bone maturing effect of gonadal estrogens and the bone compressive effect of distended lymphatics and lymphedema [9, 10, 12, 18]. In addition, it has also been suggested that shield chest is primarily ascribed to lymphstasis around the thoracic cavity, because *SHOX* is not expressed in the body trunk [19]. These findings imply that both *SHOX* haploinsufficiency and skeletal compression by distended lymphatics play a pivotal role in the development of common Turner skeletal features. Indeed, this notion would be consistent with the borderline cubitus valgus of the Turner girl examined in the present study who had *SHOX* haploinsufficiency but was free from elbow lymphstasis as well as gonadal estrogens.

This boy had several skeletal features frequently observed in both Noonan and Turner syndrome, such as high arched palate, micrognathia, shield chest, and cubitus valgus. In this regard, severe lymphstasis was delineated in the elbow region by the lymphatic scintigraphy. This would imply that the elbow lymphstasis exerted a marked compressive effect on the adjacent developing skeletal tissues, promoting the occurrence of cubitus valgus. In addition, severe lymphstasis is

also postulated in the faciocervical region by the presence of multiple facial and cervical soft tissue stigmata, although lymphatic flow was not examined for the faciocervical region. This would suggest that the faciocervical lymphstasis had a compressive effect on the neighboring skeletal tissues, facilitating the development of high arched palate and micrognathia. It is assumed, therefore, that most, if not all, skeletal features in Noonan syndrome could primarily be regarded as the deformational consequences caused by distended lymphatics and lymphedema resulting from lymphatic hypoplasia/dysplasia. However, mutations of *PTPN11* and other Noonan causative gene(s) may also have a direct deleterious effect on skeletal development. In this case, Noonan skeletal features can be ascribed to the synergistic effect of the defective

development of both skeletal and lymphatic systems.

In summary, the results imply that lymphatic hypoplasia/dysplasia is relevant to the development of not only soft tissue and visceral anomalies but also skeletal anomalies in Noonan syndrome. This hypothesis awaits further clinical studies in Noonan syndrome patients.

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