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The Role of SHOX Gene in Short Stature of Turner Syndrome and Its Variant

Tri Indah Winarni *,***, Farmaditya EP Mundhofir **,***, Sultana MH Faradz **, ***

ABSTRACT

Background: *SHOX gene is located on the edge of each short/p arm sex chromosome called the pseudoautosomal region-1 (PAR1) plays as a fundamental role on controlling chondrocyte differentiation and apoptosis in the growth plate. Longitudinal growth is determined by environmental, hormonal and genetic factors. Short stature is defined as a standing height below the third percentile according to Tanner et al. Short stature affects approximately 2% of children. Turner syndrome is the most common genetic disorder in female characterized by the absence of all or part of a normal second X chromosome, affecting 1:2500 live-born female babies. Short stature and ovarian failure is the main clinical feature. The objective of this study is to elucidate the implication of SHOX gene in short stature of Turner Syndrome and its variant.*

Method: *Purposive sampling was performed to recruit female with short stature after informed consent agreement. Female with growth treatment history and chronic diseases was excluded from this study. Cytogenetics testing was done for all samples by G-banding method, in routine karyotyping.*

Result: *We report 9 females with short stature which cytogenetically and clinically diagnosed as Turner Syndrome. Four cases is classic Turner syndrome with standing height is below third percentile, three cases are 45,X/46,X,i(Xq) with standing height is below third percentile, one case is 46,XX/45,X (80%) with standing height is below third percentile, and the rest is 46,XX/45,X (20%) with standing height is between 3^{rd}–97^{th} percentile or normal.*

Conclusion: *SHOX gene haploinsufficiency is strongly indicated the cause of short stature in Turner Syndrome.*

Key words: *SHOX gene, short stature, and Turner syndrome*

ABSTRAK

Peran gen SHOX pada perawakan pendek Sindrom Turner dan variannya

Latar belakang: *Gen SHOX terdapat di ujung lengan pendek kromosom seks yang disebut* pseudoautosomal regio-1 *(PAR1) yang berperan penting pada pengaturan diferensiasi kondrosit and apoptosis di lempeng epifisis. Pertumbuhan memanjang ditentukan oleh faktor lingkungan, hormon, dan faktor genetik. Menurut Tanner dkk, perawakan pendek didefinisikan sebagai tinggi badan kurang dari tiga persentil dan diperkirakan terjadi pada 2% populasi anak-anak. Sindrom Turner merupakan kelainan genetik pada perempuan yang paling banyak ditemukan akibat hilangnya sebagian atau seluruh kromosom X normal yang kedua dengan gambaran klinik utama berupa* short stature *dan* insufisiensi ovarium*, dengan insidensi 1:2500 bayi lahir hidup. Tujuan penelitian ini untuk memahami peran gen SHOX pada perawakan pendek Sindrom Turner dan variannya.*

Metode: *Subjek penelitian adalah wanita berperawakan pendek yang setuju mengikuti penelitian dengan menandatangani* informed consent*. Dilakukan eksklusi untuk wanita berperawakan pendek dengan riwayat pengobatan pemacu pertumbuhan dan penyakit kronik. Pemeriksaan sitogenetik dengan metode pengecatan Giemsa dilakukan pada semua preparat kromosom dilanjutkan dengan analisis kromosom rutin.*

Hasil: *Dilaporkan sembilan (9) wanita berperawakan pendek yang secara sitogenetik dan klinis didiagnosis sebagai Sindrom Turner. Empat kasus didiagnosis sebagai Sindrom Turner klasik dengan tinggi badan di bawah tiga persentil, tiga kasus dengan 45,X/46,X,i(Xq) dengan tinggi badan di bawah tiga persentil, satu kasus dengan 46,XX/45,X (80%) dengan tinggi badan di bawah tiga persentil, dan sisanya adalah 46,XX/45,X (20%) dengan tinggi badan di bawah antara 3-97 persentil atau normal.*

Simpulan: Haploinsufficiency *gen SHOX diduga kuat menyebabkan perawakan pendek pada Sindrom Turner.*

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INTRODUCTION

Turner syndrome is the most common chromosomal abnormality in female, affecting 1: 2500 live-born female.[1,2] Approximately 3% of all female fetuses affected Turner syndrome, however, 99% of them were abort spontaneously, and only 1% of them will survive. Consequently, Turner syndrome is responsible for 7-10% of all spontaneous abortions.[3] Half (50%) of them have monosomy X (45,X), 5-10% have a duplication of the long arm (isochromosome) of X chromosome (46,X,i(Xq)), and most of the rest have mosaic for 45,X, with one or more additional cell lineages.[4] There is a strong correlation between cytogenetic appearance and the phenotype in Turner syndrome. Monosomy 45,X is the most common karyotype its associated with the most abnormal phenotype, although, mosaics Turner karyotype usually have a less severe phenotype. Eighty percent of the single remaining X chromosome of Turner syndrome is inherited from the mother, and in 20% of these them its inherited from the father.[5] Monosomy X results from nondisjunction as a result of failure of the sex chromatids to separate during meiosis in the parental gamete, mosaic type usually result of mitotic nondisjunction during early embryonic division.

Short stature is a developmental and multi-factorial conditions with strongly correlates with genetic component affected on approximately 2% of children,[6] is the main characteristic of Turner syndrome due to the growth failure or premature closure of the epiphyseal growth plate.[7] The incidence is nearly 100% for short stature and 35–60% for representative skeletal anomalies such as short metacarpals, cubitus valgus, high-arched palate, micrognathia, and short neck.[8] Current evidence indicates that the genes involved in Turner syndrome are mainly located on short arm of the X chromosome called Short stature HOmeoboX-containing (SHOX) gene, a repressor of growth plate fusion and skeletal maturation, has a pivotal role in long bone elongation. This gene is located on the edge of each sex chromosome (Xpter and Ypter) in an area called the pseudoautosomal region-1 (PAR1). Female with Turner syndrome have only one copy of the SHOX gene instead of two copies, since one copy of X chromosome is absence. In female with normal karyotype, SHOX gene is expressed in both inactive and active X chromosome means that SHOX gene escape from X-inactivation. SHOX gene contains one non-coding region and six coding exons ranging from 59 to 1166bp in size which encodes a homeodomain transcription factor is believed to be responsible for the height deficit associated with Turner syndrome.[9] Haploinsufficiency, refers to the presence in the cells of 1 set of gene rather than the usual 2 set of the SHOX gene in the PAR1 of the sex chromosomes may cause short stature in Turner Syndrome.

The SHOX gene is located on the short (p) arm of the X chromosome between the end (terminus) of the arm and position 22.32 and on the short (p) arm of the Y chromosome at position 11.3. SHOX (OMIM 312865) is a single gene, a 170 kb DNA.

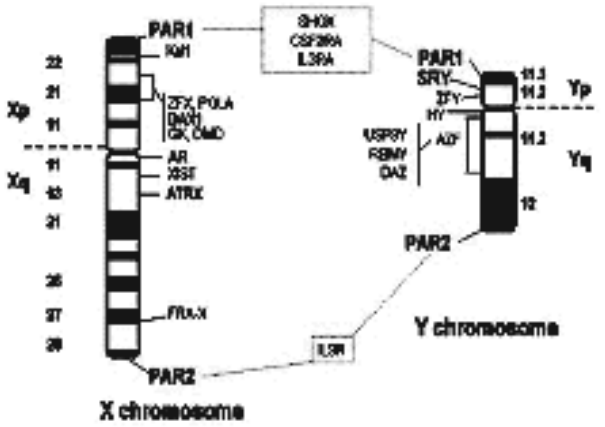

Figure 1. Location of SHOX gene on PAR1 X and Y chromosome The SHOX gene is located on pseudoautosomal region 1 (PAR 1) on the edge of short (p) arm of the X and Y chromosome containing the SHOX gene, the gene responsible for the length of the bone especially the long bone. [10]

Expression of SHOX genes have shown in human growth plate chondrocytes with the highest expression in terminally differentiated hyperthropic cells, suggesting its involvement in the process controlling chondrocyte differentiation and apoptosis in the growth plate. In human embryos the earliest evidence of SHOX gene expression was in the developing limbs from 32 day conception onward.[11] Further study proved that expression of SHOX gene in primary chondrocytes is associated with decrease of cell proliferation, differentiation, and apoptosis process in human growth plate chondrocytes.[9] Longitudinal growth takes place in the growth plate through a process called endochondral occification. The balance between proliferation and differentiation is a crucial regulatory step controlled by a number of transcription factors, hormones, and growth factors. Abnormalities in the growth plate may lead to short stature and skeletal deformity seen in many skeletal dysplasia.[12]

Endochondral ossification begins by the end of the embryonic period. Primary ossification centre are present in all long bones of the limbs by the 12th week development. From the primary center in diaphysis of the bone, endochondral ossification gradually pro-gresses toward the ends of the cartilaginous model. At the birth, the diaphysis of the bone is usually completely

ossified, but the two ends, the epiphyses, are still cartilaginous. Shortly thereafter, ossification centers arise in the epiphyses. A cartilage temporary remains between the diaphyseal and epiphyseal ossification centers on both sides, called epiphyseal plate, its plays an important role in growth in the length of the bones. When the bone has acquired full length, the epiphyseal plate dissappear and the epiphyses unite with the shaft of the bone.[13]

The role of SHOX gene as a repressor of growth plate fusion and skeletal maturation, so that SHOX haploinsufficiency results unbalanced, premature growth plate fusion, and advanced skeletal maturation.[13] Some clinical investigators reported that SHOX haploinsufficiency could lead other skeletal anomalies in Turner syndrome like short fourth metacarpal, Madelung deformity. The aim of study is to elucidate the implication haploinsufficiency of SHOX gene in female with short stature.

METHODS

Female were diagnosed Turner syndrome based on both clinical and cytogenetic included in this research. Cross-sectional research design conducted in 9 females with Turner syndrome. Stature measurement was performed according to Tanner Female Linier Standard. Subject were categorized as short stature when height below the third percentile.[14] Patient and family (when the subject has not matured enough to make their own decision) were recruited after informed concent agreement. Female with growth treatment history and chronic diseases was excluded from this study.

Cytogenetics testing was done for all samples by Giemsa-banding method, in routine karyotyping, 20 cells were counted. If it is more than one cells line were found (mosaicism), 100 cells were counted. Karyotyping of a blood samples is definitive in most cases.

Data were analyzed using Fisher exact risk calculation.

RESULT

Among 9 subjects 8 patients were categorized as short stature (88.9%) and 1 as normal stature (11.1%). Cytogenetics analysis show 7 subjects (77.8%) were haploinsufficiency due to lost of p arm of X chromosome and 2 subjects was mozaic haploinsufficiency (22.2%). Karyotype analysis is shown on Table 1.

Using Giemsa-banding cytogenetic analysis, it's representing all female with short stature confirmed as Turner syndrome classic or variants type. Karyotype of variant Turner syndrome (subject no 9) describes bellow:

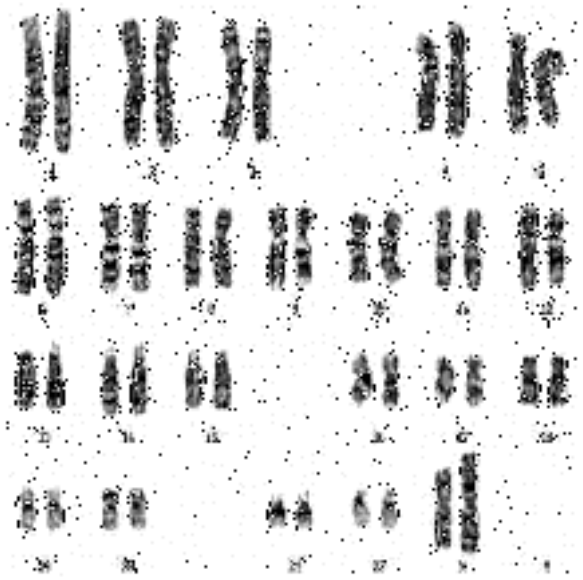

Figure 2. Representative Giemsa-banding karyotyping of Turner syndrome with 46,X,I (Xq). Isochromosome q arm of X chromosome indicate the cells contain only long arm chromosome, means that there is haploinsufficiency of SHOX gene located on PAR1 (p arm of X chromosome).

Risk analysis yield prevalence ratio of haploinsufficiency to have short stature was 2. (95% CI=0.5 to 8.0) statistically not significant. This analysis show

Table 1. Representing the age, karyotype result, the height, and tanner female linier standard of height

Subject	Karyotype	Height (cm)	Female Linier Standard
1.	45,X	130	< 3rd percentile
2.	45,X	130	< 3rd percentile
3.	45,X	132	< 3rd percentile
4.	45,X/46,X,i(Xq) (25%)	91	< 3rd percentile
5.	46,XX/45X (20%)	156	3rd-97th percentile
6.	45,X/46,X,i(Xq) (1%)	144	< 3rd percentile
7.	46,XX/46,X (80%)	118	< 3rd percentile
8.	45,X	147	< 3rd percentile
9.	46,Xi(Xq)(85%)/45,X(15%)	137	< 3rd percentile

Table 2. Distribution of SHOX gene according stature categories

SHOX gene	Stature		Total
	Short	Normal	
Haploinsufficiency	7 (77.8%)	0 (0.0%)	7 (77.8%)
Mozaic	1 (11.1%)	1 (11.1%)	2 (22.2%)
Total	8 (78.9%)	1 (11.1%)	9 (100%)

Fisher exact analysis: p=0,222.

subjects with haploinsufficiency had risk to have short stature 2 times higher than mosaic subjects. However, 95% CI across 1 therefore haploinsufficiency can be concluded as risk factor. Since upper bound 95% CI was 8.0, haploinsufficiency tend to be risk factor of short stature. Further study with big sample size is necessary to determine the association between haploinsufficiency with short stature.

DISCUSSION

Growth retardation resulting in short stature is a major concern for parents and due to its great variety of causes. SHOX gene is located on PAR1 on each human short arm of sex chromosome (Xp22 or Yp11.3); a major locus involved in linear growth has been proved.

Most women with Turner syndrome have only one copy of the SHOX gene instead of two copies, haploinsufficiency, which reduces the amount of SHOX protein by a half. The loss of action of the one allele result in a reduction of 50% overall activity. Regarding many studies before, haploinsufficiency of SHOX gene is associated with increasing of cell proliferation, differentiation, and apoptosis process in human growth plate chondrocytes that responsible to the premature of growth plate closure.[7]

According to Tanner et al Female Linier Standard, eight cases confirm as a short stature (below the third percentile), while one case has a normal height (3^{rd}–97^{th} percentile). Regarding to their karyotype, cases no. 1, 2, 3, and 8 are confirm as monosomy Turner syndrome (45,X), case no. 7 is mosaic 46,XX/46,X (80%) means that most of the cells contains only one copy of SHOX gene because most of the cells contains monosomy 45,X. Case no. 4 is mosaic 45,X/46,X,i(Xq) (25%), no. 6 is mosaic 45,X/46,X,i(Xq) (1%) and cases no. 9 is mosaic 46,Xi(Xq)(85%)/45,X (15%). In patient with karyotype 46,X,Xi(Xq) contains 44 chromosome autosome, one normal chromosome X, and one abnormal chromosome X which is the long arm (q) is duplicated and the short arm (p) is lost, means that all of the cells contains only one SHOX gene. We can conclude that they only have one copy of SHOX gene in PAR1 of X chromosome instead of two, called SHOX gene haploinsufficiency. Therefore, only half of homeodomain plays as a fundamental role on controlling chondrocyte differentiation and apoptosis in the growth plate. These conditions may cause premature of growth plate fusion resulting short stature. Case no. 5 is a mosaic Turner syndrome with 46,XX/45,X (20%), it means that only 20% of cells contains monosomy 45,X and mostly cells contains two copy of SHOX gene in PAR1 of both X chromosome. Two copy of SHOX gene expresses in primary chondrocytes controlling cell proliferation, differentiation, and apoptosis process in human growth plate chondrocytes, it is required the bone to reach full length. Our data displays on Table 1 confirm with many studies.

CONCLUSION

Sex chromosome abnormality (monosomy) of numerical and or structural involving the short arm chromosome is strongly indicated the cause of short stature in Turner Syndrome. Subjects with happloinsufficiency tend to have short stature 2 times higher than subjects with mosaic karyotype. SHOX gene haploinsufficiency is strongly indicated the cause of short stature in Turner Syndrome.

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