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ORIGINAL RESEARCH

Associations of Karyotype and Age at Diagnosis with Physical Features and Comorbidities in Turner Syndrome: A Single-Site Experience

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Aim: Turner syndrome (TS) is one of the most common genetic diseases in females, with typical physical features and comorbidities. Karyotype-phenotype associations and clinical significance of childhood versus adolescent/adulthood diagnosis are conflicting.

Purpose: Determining the role of certain TS karyotypes and early (<12 years of age) vs late (≥12 years) diagnosis in TS-specific phenotype and comorbidity penetrance.

Patients and Methods: Retrospective analysis of baseline characteristics and 45 TS-specific features and comorbidities of 75 TS patients were diagnosed between 2009 and 2019 and followed-up until 2023 in our tertiary care center.

Results: Thirteen different karyotypes were detected: 45,X,inv(10), 45,X,inv(9)(15), 45,X, 46,X,i(Xq), 46,X,del(Xp), 46,XX,del(X)q21, 45,X/46,X,del(X), 45,X/46,X,+mar, 45,X/46,X,rX, 45,X/46,XX, 45,X/46,XY, 45,X/47,XXX, 46,X,i(Xq)/47,XX,i(Xq). The classic karyotype with 45X monosomy showed an increased risk for hypertrichosis (28.6% vs 7.5%, OR 4.93, 95% CI [1.23–19.73]), pterygium colli (34% vs 12%, OR 3.65, 95% CI [1.13–11.75]) and short stature (91% vs 75%, OR 3.56 [0.89–14.17]. Mosaic karyotypes had a smaller risk of pterygium colli (OR 0.28 [0.073–1.092]) and short stature (OR 0.29 [0.086–1.026]. 45X/46XX mosaicism was associated with an increased risk of hypertension (33% vs 6%, OR 7.75 [1.39–43.08]), and the presence of the iso (Xq) chromosome increased the risk of celiac disease (28% vs 3%, OR 13.2 [1.52–114.52]). 44/75 (58.6%) of the cohort were diagnosed at <12 years of age. In the <12-year-old diagnosis group, facial dysmorphism and low hairline, (OR 3.30, [1.26–8.65]), low-set ears (OR 2.51 [0.98–6.46]), and breasts abnormalities (OR 4.71 [1.72–12.83]), short stature (OR 4.09 [1.13–14.82]) and GH therapy (OR 4.93 [1.31–16.01]) occurred more frequently. If diagnosed <12 years, patients had a decreased risk of hepatosplenomegaly (OR 0.10 [0.02–0.50]) and hypertension (OR 0.097 [0.01–0.85]).

Conclusion: TS patients should be handled as a heterogenous group, as they seem to differ in the penetrance of phenotypical features of the disease and the risk of comorbidities depending on karyotype and age at diagnosis.

Keywords: turner-syndrome, karyotype-phenotype association, comorbidities, mosaicism, incomplete penetrance, premature ovarian insufficiency

Introduction

Turner syndrome (TS) is a genetic disorder caused by the monosomy of the X chromosome or structural alterations of the X or Y chromosomes. It is one of the most frequent genetic aneuploidies affecting 1 in every 2500 live-born females.[1,2] Among other physical features, short stature and gonadal failure caused delayed puberty are characteristics of the disease, along with epicanthic folds, down slanting palpebral fissures, low set ears, micrognathia or pigmented naevi.[3,4] Cardiovascular, gynecologic, endocrine, gastroenterologic, neuropsychiatric and autoimmune disorders occur more frequently in TS patients as compared to the general population, and these need to be diagnosed and treated as soon as possible, as well as followed-up throughout the entire life span.[5] Nevertheless, TS is not a homogenous disease: the penetrance of the classical features (short stature, breast anomaly,

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hypertrichosis, low hairline, flat face, pterygium colli, low set ears, striae, acanthosis, or hypoplastic vagina) or the comorbidities (eg kidney malformations, hypertension, tachycardia, aortic stenosis, mitral valve prolapse, tricuspid valve insufficiency, atrial septum defect, coarctation of the aorta, patent ductus arteriosus, aortic insufficiency etc.) seem to be different depending on the genetic background, ie, the specific karyotype, or whether the non-mosaic or mosaic form of the disease is present.[5] Karyotype-phenotype associations suggest that clinical phenotype may be determined by the expression and amount of proteins coded by of the X chromosome genes. Haploinsufficiency of the Xp arm, increased dosage of the Xq arm,[6] or the percentage of the cell lines that bear a modified karyotype in mosaic forms all may have an effect on the final phenotype.[7,8]

The best known cytogenetic finding is the classical 45,X, but a broad spectrum of other karyotypes can also cause TS phenotype with full or partial penetrance, such as 45,X/46,XX and other mosaicisms, structural rearrangements, for example isochromosome of the long arm of the X chromosome [46,X,i(Xq)], ring chromosomes like 46Xr(X), deletions like 46,X,del(Xp), or complete/partial lack of the Y chromosome.[1,9] Although TS patients with mosaicism or non-classical karyotypes are reported to have the phenotypical features less emphasized,[6–8] their clinical management needs to correspond with that of the classical 45,X cases, because TS related complications may be asymptomatic for a long time. In the young adult age, reproduction is often in the focus, but comorbidities are crucial to be addressed properly at this age, too. TS patients may ovulate and can get pregnant spontaneously, but it is more common that they are able to get pregnant using assisted reproduction such as oocyte donation and in vitro fertilization (IVF). This requires well-planned prenatal care with mandatory cardiovascular and endocrine follow-ups, to prevent potentially life-threatening complications during pregnancy or peripartum.[10] Beyond the scope of reproduction, specialists are often confronted with various difficulties during the long-term care of TS patients – one of the hardest steps being the transition from pediatric to adult care because of the disappearance of the patients during medical care.[11] Therefore, it is not always possible to have compliant patients and perform a full-scale diagnostic or screening medical check-up, and, furthermore, in some regions at certain times health-care resources can be limited. Thus, it is important to focus on the most common, probable, or dangerous comorbidities related to TS, which then can be taken care of by specialists of the field.[12]

The aim of our investigation was to collect detailed data of the patients diagnosed and treated with TS at the pediatric and gynecologic endocrine units of our tertiary level university hospital. We looked for associations between the age at diagnosis and specific karyotypes or karyotype subgroups (classical, non-classical, mosaic, non-mosaic) and physical TS features, as well as comorbidities, in order to improve the follow-up strategy of TS patients.

Materials and Methods

Patients and Data Collection

In our retrospective analysis, we collected data from our Turner syndrome database of 75 patients diagnosed with Turner syndrome at the Department of Obstetrics and Gynaecology and the Department of Pediatrics, University of Debrecen, between 1 January 2009 and 1 June 2019. Follow-up was included in the study until 1 June 2023. All TS patients diagnosed and followed up in the given period were included and analysed in possession of patient consent. Data were collected from hospital charts and the local (Med Solution, UDMed) and the Hungarian national (EESZT, Elektronikus Egészségügyi Szolgáltatási Tér, Electronic Healthcare Providers' System) electronic medical databases, including in- and outpatient reports of gynaecologic, paediatric, neonatologic, endocrinologic, cardiologic, internal medicine, urologic, psychiatric, otolaryngologic, ophthalmologic, neurologic, dermatologic and genetic consultations, imaging and laboratory results (ethical approval: DE RKEB/IKEB 5953-2022).

Examinations and Screening Strategy

Diagnosis was confirmed by karyotyping in each case using peripheral lymphocytes from blood test. All TS patients were screened for comorbidities after the diagnosis was proved, in accordance with local protocols, which adapt the recommendations of the International Turner Syndrome Consensus Group published in 2017.[13] 45 variables (baseline characteristics, physical features of TS, growth disorder, gynecologic, cardiologic, endocrinologic, gastroenterologic diseases, and diseases regarding the sensory organs, mental health, and kidneys) were recorded. The specific features and comorbidities screened and analysed are those listed in Tables 1–5. Baseline characteristics and physical Turner

Table 1 Baseline Characteristics, Physical Features and Comorbidities of the Whole Study Cohort and the Mosaic and Non-Mosaic Subgroups

Baseline characteristics	**All (n=75)**		**Mosaic (n=28)**		**Non-mosaic (n=47)**		**Mosaic vs Non-mosaic**		
	Mean	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	**p**		
Age at diagnosis (y)	9.6	(5.8)	9.4	(6.4)	9.7	(5.5)	0.825		
Height at diagnosis (cm)	127.1	(22.1)	126.5	(23.4)	127.4	(21.5)	0.921		
Weight at diagnosis (kg)	41.3	(20.5)	39.4	(18.5)	42.5	(21.0)	0.751		
BMI at diagnosis (kg/m2)	24.7	(7.6)	22.6	(6.1)	26.0	(8.1)	0.269		
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	**OR**	**(95% CI)**	**p**
Early diagnosis (<12y)	44	(58.7)	18	(64.3)	26	(55.3)	1.45	0.555–3.810	0.446
Growth disorder									
Short stature	62	(82.7)	20	(71.4)	42	(89.4)	**0.30**	**0.086–1.026**	**0.055**
GH therapy	58	(77.3)	22	(78.6)	36	(76.6)	1.12	0.363–3.459	0.843
Turner phenotype									
Breast abnormality**	47	(62.7)	18	(64.3)	29	(61.7)	1.12	0.423–2.951	0.823
Hypertrichosis	13	(17.3)	0	(0.0)	**13**	**(27.7)**	**n/a**		
Low hairline, flat face	44	(58.7)	16	(57.1)	28	(59.6)	0.91	0.350–2.336	0.836
Pterygium colli	17	(22.7)	3	(10.7)	14	(29.8)	**0.28**	**0.073–1.092**	**0.067**
Low set, prominent ears	39	(52.0)	15	(53.6)	24	(51.1)	1.11	0.433–2.823	0.833
Striae	14	(18.7)	3	(10.7)	11	(23.4)	0.39	0.099–1.553	0.183
Acanthosis, naevi	19	(25.3)	9	(32.1)	10	(21.3)	1.75	0.609–5.043	0.298
Gynecology									
Hypoplastic vagina	4	(5.3)	1	(3.6)	3	(6.4)	0.543	0.054–5.491	0.605
Abnormal pelvic US*	37	(49.3)	11	(39.3)	26	(55.3)	0.52	0.202–1.354	0.181
Clitoromegaly	4	(5.3)	1	(3.6)	3	(6.4)	0.54	0.054–5.491	0.605
Cardiac									
Hypertension	7	(9.3)	3	(10.7)	4	(8.5)	1.29	0.267–6.238	0.751
Tachycardia	10	(13.3)	4	(14.3)	6	(12.8)	1.14	0.292–4.445	0.852
Aortic stenosis	4	(5.3)	2	(7.1)	2	(4.3)	1.73	0.230–13.028	0.594
Mitral valve prolapse	9	(12.0)	6	(21.4)	3	(6.4)	**4.00**	**0.913–17.526**	**0.066**
Tricuspid valve insufficiency	1	(1.3)	0	(0.0)	1	(2.1)	n/a		
Atrial septum defect	9	(12.0)	3	(10.7)	6	(12.8)	0.82	0.188–3.575	0.792
Coarctation of the aorta	3	(4.0)	1	(3.6)	2	(4.3)	0.83	0.072–9.632	0.844
Patent ductus arteriosus	3	(4.0)	3	(10.7)	0	(0.0)	n/a		
Aortic insufficiency	4	(5.3)	1	(3.6)	3	(6.4)	0.54	0.054–5.491	0.605

(*Continued*)

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Table 1 (Continued).

Endocrinology, metabolism									
Obesity	17	(22.7)	5	(17.9)	12	(25.5)	0.63	0.197–2.040	0.445
Type 1 diabetes mellitus	6	(8.0)	2	(7.1)	4	(8.5)	0.83	0.141–4.835	0.833
Hypothyroidism	36	(48.0)	15	(53.6)	21	(44.7)	1.43	0.558–3.655	0.457
Hyperthyroidism	2	(2.7)	0	(0.0)	2	(4.3)	n/a		
Hashimoto thyroiditis	14	(18.7)	6	(21.4)	8	(17.0)	1.33	0.408–4.329	0.636
Gastroenterology									
Celiac disease	4	(5.3)	0	(0.0)	4	(8.5)	n/a		
Ulcerative colitis	3	(4.0)	1	(3.6)	2	(4.3)	0.83	0.072–9.632	0.884
Hepatosplenomegaly	12	(16.0)	6	(21.4)	6	(12.8)	1.86	0.537–6.469	0.327
Sensory functions									
Anosmia	1	(1.3)	1	(3.6)	0	(0.0)	n/a		
Hypacusis	13	(17.3)	5	(17.9)	8	(17.0)	1.06	0.310–3.627	0.926
Tympanoplasty	4	(5.3)	3	(10.7)	1	(2.1)	5.52	0.545–55.888	0.148
Ophthalmologic disorder***	20	(26.7)	7	(25.0)	13	(27.7)	0.87	0.300–2.536	0.801
Mental health									
Depression	8	(10.7)	2	(7.1)	6	(12.8)	0.53	0.099–2.804	0.451
Suicide	1	(1.3)	1	(3.6)	0	(0.0)	n/a		
Epilepsy	3	(4.0)	1	(3.6)	2	(4.3)	0.83	0.072–9.632	0.884
Kidney diseases									
Kidney stone	1	(1.3)	0	(0.0)	1	(2.1)	n/a		
Hydronephrosis	4	(5.3)	1	(3.6)	3	(6.4)	0.54	0.054–5.491	0.605
Hypoplastic kidney	2	(2.7)	0	(0.0)	2	(4.3)	n/a		
Horseshoe kidney	2	(2.7)	0	(0.0)	2	(4.3)	n/a		
Pyelectasis	2	(2.7)	1	(3.6)	1	(2.1)	1.70	0.102–28.362	0.71

Notes: *Streak gonad, hypoplastic uterus, atrophic endometrium, **Widely spaced nipples, underdeveloped breasts, ***Strabism, astigmatism, hypermetropy. Bold indicates significant results (p<.,05) or statistically non-significant trends (0.05<p<0.10).
Abbreviation: US, ultrasound.

syndrome features, as well as blood pressure were determined during the physical examination at the time of diagnosis. After the confirmation of TS, echocardiography and abdominal (renal) ultrasound, audiometry, ophthalmologic examination, thyroid function testing by TSH, fT4, thyroid autoimmunity screening by anti-TPO, diabetes screening by HbA1c, and celiac disease screening by anti-tTG examination were performed. In accordance with international recommendations, examinations were repeated every 1 to 5 years, the frequency depending on the disease tested and the results of the initial examinations. Genitalia were assessed by gynaecologic examination and/or ultrasonography, abnormal findings defined as having streak gonads, hypoplastic uterus or atrophic endometrium. Psychiatric examination was only performed if symptoms of mental health disorders appeared. Obesity was defined as BMI >30 kg/m2. Growth was

Table 2 Baseline Characteristics, Physical Features, and Comorbidities of the Subgroups of 45,X and the Pooled Other Karyotypes

Baseline characteristics	**45,X (n=35)**		**Other (n=40)**					
	Mean	**(SD)**	**Mean**		**(SD)**	**p**		
Age at diagnosis (y)	9.5	(5.8)	8.8		(5.3)	**0.001**		
Height at diagnosis (cm)	128.7	(21.3)	124.5		(22.2)	**0.003**		
Weight at diagnosis (kg)	43.4	(22.7)	39.4		(20.5)	**0.006**		
BMI at diagnosis (kg/m2)	25.9	(8.8)	24.6		(8.0)	0.192		
	n	**(%)**	**n**	**(%)**	**(%)**	**OR**	**(95% CI)**	**p**
Early diagnosis (<12y)	19	(54.3)	25	0.625	(62,5)	0.71	(0.28–1.79)	0.472
Growth disorder								
Short stature	32	(91.4)	30	0.750	(75.0)	**3.56**	**(0.89–14.17)**	**0.072**
GH therapy	28	(80.0)	30	0.750	(75.0)	1.33	(0.44–3.98)	0.606
Turner phenotype								
Breast abnormality**	23	(65.7)	24	0.600	(60.0)	1.28	(0.49–3.27)	0.61
Hypertrichosis	10	(28.6)	3	0.075	(7.5)	**4.93**	**(1.23–19.73)**	**0.02**
Low hairline, flat face	22	(62.9)	22	0.550	(55.0)	1.39	(0.54–3.49)	0.49
Pterygium colli	12	(34.3)	5	0.125	(12.5)	**3.65**	**(1.13–11.74)**	**0.03**
Low set, prominent ears	19	(54.3)	20	0.500	(50.0)	1.19	(0.47–2.94)	0.711
Striae	8	(22.9)	6	0.150	(15.0)	1.68	(0.52–5.42)	0.387
Acanthosis, naevi	7	(20.0)	12	0.300	(30.0)	0.58	(0.20–1.69)	0.323
Gynecology								
Hypoplastic vagina	3	(8.6)	1	0.025	(2.5)	3.66	(0.36–36.87)	0.272
Abnormal pelvic US*	21	(60.0)	16	0.400	(40.0)	**2.25**	**(0.89–5.68)**	**0.086**
Clitoromegaly	3	(8.6)	1	0.025	(2.5)	3.66	(0.36–36.87)	0.272
Cardiac								
Hypertension	4	(11.4)	3	0.075	(7.5)	1.59	(0.33–7.65)	0.562
Tachycardia	5	(14.3)	5	0.125	(12.5)	1.17	(0.30–4.42)	0.821
Aortic stenosis	1	(2.9)	3	0.075	(7.5)	0.36	(0.03–3.65)	0.390
Mitral valve prolapse	3	(8.6)	6	0.150	(15.0)	0.53	(0.12–2.30)	0.398
Tricuspid valve insufficiency	1	(2.9)	0	0.000	(0.0)	n/a		
Atrial septum defect	6	(17.1)	3	0.075	(7.5)	2.55	(0.58–11.08)	0.211
Coarctation of the aorta	2	(5.7)	1	0.025	(2.5)	2.36	(0.20–27.24)	0.490
Patent ductus arteriosus	0	(0.0)	3	0.075	(7.5)	n/a		
Aortic insufficiency	3	(8.6)	1	0.025	(2.5)	3.66	(0.36–36.87)	0.272

(*Continued*)

Table 2 (Continued).

Endocrinology, metabolism								
Obesity	8	(22.9)	9	0.225	(22.5)	1.02	(0.34–3.01)	0.971
Type 1 diabetes mellitus	4	(11.4)	2	0.050	(5.0)	2.45	(0.42–14.28)	0.319
Hypothyroidism	17	(48.6)	19	0.475	(47.5)	1.04	(0.42–2.58)	0.926
Hyperthyroidism	1	(2.9)	1	0.025	(2.5)	1.15	(0.06–19.04)	0.924
Hashimoto thyroiditis	7	(20.0)	7	0.175	(17.5)	1.18	(0.36–3.76)	0.782
Gastroenterology								
Celiac disease	2	(5.7)	2	0.050	(5.0)	1.15	(0.15–8.63)	0.891
Ulcerative colitis	1	(2.9)	2	0.050	(5.0)	0.56	(0.04–6.44)	0.641
Hepatosplenomegaly	6	(17.1)	6	0.150	(15.0)	1.17	(0.34–4.03)	0.801
Sensory functions								
Anosmia	0	(0.0)	1	0.025	(2.5)	n/a		
Hypacusis	6	(17.1)	7	0.175	(17.5)	0.98	(0.29–3.23)	0.967
Tympanoplasty	1	(2.9)	3	0.075	(7.5)	0.36	(0.03–3.65)	0.390
Ophthalmologic disorder***	10	(28.6)	10	0.250	(25.0)	1.20	(0.43–3.34)	0.727
Mental health								
Depression	6	(17.1)	2	0.050	(5.0)	3.93	(0.73–20.91)	0.109
Suicide	0	(0.0)	1	0.025	(2.5)	n/a		
Epilepsy	2	(5.7)	1	0.025	(2.5)	2.36	(0.20–27.24)	0.490
Kidney diseases								
Kidney stone	1	(2.9)	0	0.000	(0.0)	n/a		
Hydronephrosis	2	(5.7)	2	0.050	(5.0)	1.15	(0.15–8.63)	0.891
Hypoplastic kidney	2	(5.7)	0	0.000	(0.0)	n/a		
Horseshoe kidney	2	(5.7)	0	0.000	(0.0)	n/a		
Pyelectasis	1	(2.9)	1	0.025	(2.5)	1.15	(0.06–19.04)	0.924

Notes: *Streak gonad, hypoplastic uterus, atrophic endometrium, **Widely spaced nipples, underdeveloped breasts, ***Strabism, astigmatism, hypermetropy. Bold indicates significant results ($p<0.05$) or statistically non-significant trends ($0.05<p<0.10$).
Abbreviation: US, ultrasound.

highly followed and short stature was declared if the height of the girl fell below the 3rd percentile of the normal female growth chart, and in each such case recombinant growth hormone (rGH) therapy was offered.

Karyotyping

Samples were evaluated at the Cytogenetics Laboratory, Department of Obstetrics and Gynaecology, University of Debrecen. Cytogenetic examination in prenatal care was performed from the sample obtained during amniotic fluid sampling and confirmed after birth from peripheral blood lymphocytes. If the primary testing occurred postnatally, karyotyping was carried out from peripheral lymphocytes. After a short cell culture (72 hours), the analysis of chromosome stock was examined using G-banding, with this technique an average of 10–15 cells could be checked,

Table 3 Baseline Characteristics, Physical Features and Comorbidities of the Subgroups Containing and Lacking (Xq) Isochromosome

Baseline characteristics	Iso(Xq) present vs Iso(Xq) absent							Iso(Xq) present vs 45,X		
	Iso(Xq) present (n=7)		Iso(Xq) absent (n=68)					45,X (n=7)		
	Mean	(SD)	Mean	(SD)	p			Mean	(SD)	p
Age at diagnosis (y)	10.0	5.0	9.5	5.9	0.85			9.5	(5.8)	0.83
Height at diagnosis (cm)	115.4	21.9	115.4	21.9	0.18			115.4	(21.3)	0.18
Weight at diagnosis (kg)	31.7	14.3	42.3	20.4	0.18			43.4	(22.7)	0.19
BMI at diagnosis (kg/m2)	23.4	12.0	24.9	7.1	0.63			25.9	(8.8)	0.59
	n	(%)	n	(%)	OR	(95% CI)	p	n	(%)	p
Early diagnosis (<12y)	5	(71.4)	39	(57.4)	1.86	0.337–10.266	0.477	19	(54.3)	0.68
Growth disorder										
Short stature	6	(85.7)	56	(82.4)	1.29	0.141–11.684	0.823	32	(91.4)	0.53
GH therapy	6	(85.7)	52	(76.5)	1.85	0.207–16.494	0.583	28	(80.0)	0.59
Turner phenotype										
Breast abnormality**	4	(57.1)	43	(63.2)	0.78	0.160–3.749	0.751	23	(65.7)	0.68
Hypertrichosis	1	(14.3)	12	(17.6)	0.78	0.086–7.068	0.823	10	(28.6)	0.65
Low hairline, flat face	4	(57.1)	40	(58.8)	0.93	0.194–4.499	0.931	22	(62.9)	0.55
Pterygium colli	1	(14.3)	16	(23.5)	0.54	0.061–4.839	0.583	12	(34.3)	0.40
Low set, prominent ears	2	(28.6)	37	(54.4)	0.34	0.061–1.849	0.210	19	(54.3)	0.41
Striae	1	(14.3)	13	(19.1)	0.71	0.078–6.374	0.756	8	(22.9)	0.53
Acanthosis, naevi	2	(28.6)	17	(25.0)	1.20	0.213–6.764	0.836	7	(20.0)	0.63
Gynecology										
Hypoplastic vagina	0	(0.0)	4	(5.9)	n/a			3	(8.6)	1.0
Abnormal pelvic US*	2	(28.6)	35	(51.5)	0.38	0.068–2.080	0.263	21	(60.0)	0.21
Clitoromegaly	0	(0.0)	4	(5.9)	n/a			3	(8.6)	1.0
Cardiac										
Hypertension	0	(0.0)	7	(10.3)	n/a			4	(11.4)	1.0
Tachycardia	0	(0.0)	10	(14.7)	n/a			5	(14.3)	1.0
Aortic stenosis	0	(0.0)	4	(5.9)	n/a			1	(2.9)	1.0
Mitral valve prolapse	1	(14.3)	8	(11.8)	1.25	0.133–11.763	0.845	3	(8.6)	0.53
Tricuspid valve insufficiency	0	(0.0)	1	(1.5)	n/a			1	(2.9)	1.0
Atrial septum defect	0	(0.0)	9	(13.2)	n/a			6	(17.1)	0.57
Coarctation of the aorta	0	(0.0)	3	(4.4)	n/a			2	(5.7)	1.0
Patent ductus arteriosus	0	(0.0)	3	(4.4)	n/a			0	(0.0)	1.0
Aortic insufficiency	0	(0.0)	4	(5.9)	n/a			3	(8.6)	1.0

(*Continued*)

Table 3 (Continued).

Endocrinology, metabolism										
Obesity	2	(28.6)	15	(22.1)	1.41	0.249–8.029	0.696	8	(22.9)	0.98
Type 1 diabetes mellitus	0	(0.0)	6	(8.8)	n/a			4	(11.4)	1.0
Hypothyroidism	4	(57.1)	32	(47.1)	1.50	0.312–7.216	0.613	17	(48.6)	1.0
Hyperthyroidism	0	(0.0)	2	(2.9)	n/a			1	(2.9)	1.0
Hashimoto thyroiditis	0	(0.0)	14	(20.6)	n/a			7	(20.0)	0.33
Gastroenterology										
Celiac disease	2	(28.6)	2	(2.9)	**13.20**	**1.521–114.521**	**0.019**	2	(5.7)	0.12
Ulcerative colitis	0	(0.0)	3	(4.4)	n/a			1	(2.9)	1.0
Hepatosplenomegaly	0	(0.0)	12	(17.6)	n/a			6	(17.1)	0.57
Sensory functions										
Anosmia	0	(0.0)	1	(1.5)	n/a			0	(0.0)	n/a
Hypacusis	1	(14.3)	12	(17.6)	0.78	0.086–7.068	0.823	6	(17.1)	1.0
Tympanoplasty	0	(0.0)	4	(5.9)	n/a			1	(2.9)	1.0
Ophthalmologic disorder***	2	(28.6)	18	(26.5)	1.11	0.198–6.243	0.905	10	(28.6)	1.0
Mental health										
Depression	0	(0.0)	8	(11.8)	n/a			6	(17.1)	0.56
Suicide	0	(0.0)	1	(1.5)	n/a			0	(0.0)	n/a
Epilepsy	0	(0.0)	3	(4.4)	n/a			2	(5.7)	1.0
Kidney diseases										
Kidney stone	0	(0.0)	1	(1.5)	n/a			1	(2.9)	1.0
Hydronephrosis	0	(0.0)	4	(5.9)	n/a			2	(5.7)	1.0
Hypoplastic kidney	0	(0.0)	2	(2.9)	n/a			2	(5.7)	1.0
Horseshoe kidney	0	(0.0)	2	(2.9)	n/a			2	(5.7)	1.0
Pyelectasis	0	(0.0)	2	(2.9)	n/a			1	(2.9)	1.0

Notes: *Streak gonad, hypoplastic uterus, atrophic endometrium, **Widely spaced nipples, underdeveloped breasts, ***Strabism, astigmatism, hypermetropy. Bold indicates significant results ($p<0.05$) or statistically non-significant trends ($0.05<p<0.10$).
Abbreviation: US, ultrasound.

during the technique Giemsa staining was used. In case of further analysis, several cells (50–100) and smaller structural chromosomal abnormalities could be examined using FISH technique (fluorescence in situ hybridization) to examine microdeletions.

Statistical Analysis

To analyse the role of certain karyotypes, comparisons were made between the classic karyotype with 45,X monosomy and other karyotypes; all mosaic and all non-mosaic karyotypes; karyotypes containing the iso (Xq) or the ring(X) rearranged chromosome structures versus those that do not. As we hypothesized that the early (ie, before the average age of menarche, at <12 years) and late (after the average age of menarche, at ≥12 years) diagnosis groups might differ in the

Table 4 Baseline Characteristics, Physical Features and Comorbidities of the Subgroups Containing and Lacking Ring(X) Chromosome

Baseline characteristics	**Ring(X) present (n=7)**		**Ring(X) absent (n=68)**				
	Mean	**(SD)**	**Mean**	**(SD)**	**p**		
Age at diagnosis (y)	9.0	5.2	9.6	5.9	0.76		
Height at diagnosis (cm)	120.2	29.1	127.8	21.4	0.461		
Weight at diagnosis (kg)	37.0	21.1	41.8	20.0	0.306		
BMI at diagnosis (kg/m2)	24.2	7.9	24.8	7.6	0.884		
	n	**(%)**	**n**	**(%)**	**OR**	**(95% CI)**	**p**
Early diagnosis (<12y)	5	(71.4)	39	(57.4)	1.86	0.337–10.266	0.477
Growth disorder							
Short stature	4	(57.1)	58	(85.3)	**0.23**	**0.045–1.186**	**0.079**
GH therapy	6	(85.7)	52	(76.5)	1.85	0.207–16.494	0.583
Turner phenotype							
Breast abnormality**	5	(71.4)	42	(61.8)	1.55	0.28–8.567	0.617
Hypertrichosis	0	(0.0)	13	(19.1)	n/a		
Low hairline, flat face	6	(85.7)	38	(55.9)	4.74	0.541–41.505	0.160
Pterygium colli	0	(0.0)	17	(25.0)	n/a		
Low set, prominent ears	5	(71.4)	34	(50.0)	2.50	0.453–13.786	0.293
Striae	1	(14.3)	13	(19.1)	0.71	0.078–6.374	0.756
Acanthosis, naevi	3	(42.9)	16	(23.5)	2.44	0.493–12.053	0.275
Gynecology							
Hypoplastic vagina	0	(0.0)	4	(5.9)	n/a		
Abnormal pelvic US*	4	(57.1)	33	(48.5)	1.41	0.294–6.802	0.665
Clitoromegaly	1	(14.3)	3	(4.4)	3.61	0.323–40.316	0.297
Cardiac							
Hypertension	0	(0.0)	7	(10.3)	n/a		
Tachycardia	1	(14.3)	9	(13.2)	1.09	0.117–10.163	0.938
Aortic stenosis	0	(0.0)	4	(5.9)	n/a		
Mitral valve prolapse	0	(0.0)	9	(13.2)	n/a		
Tricuspid valve insufficiency	0	(0.0)	1	(1.5)	n/a		
Atrial septum defect	0	(0.0)	9	(13.2)	n/a		
Coarctation of the aorta	0	(0.0)	3	(4.4)	n/a		
Patent ductus arteriosus	1	(14.3)	2	(2.9)	5.50	0.433–69.862	0.189
Aortic insufficiency	0	(0.0)	4	(5.9)	n/a		

(*Continued*)

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Table 4 (Continued).

Endocrinology, metabolism							
Obesity	3	(42.9)	14	(20.6)	2.89	0.579–14.447	0.195
Type 1 diabetes mellitus	1	(14.3)	5	(7.4)	2.10	0.210–21.041	0.528
Hypothyroidism	5	(71.4)	31	(45.6)	2.98	0.541–16.462	0.210
Hyperthyroidism	0	(0.0)	2	(2.9)	n/a		
Hashimoto thyroiditis	2	(28.6)	12	(17.6)	1.87	0.323–10.789	0.486
Gastroenterology							
Celiac disease	0	(0.0)	4	(5.9)	n/a		
Ulcerative colitis	0	(0.0)	3	(4.4)	n/a		
Hepatosplenomegaly	1	(14.3)	11	(16.2)	0.86	0.094–7.898	0.897
Sensory functions							
Anosmia	0	(0.0)	1	(1.5)	n/a		
Hypacusis	1	(14.3)	12	(17.6)	0.78	0.086–7.068	0.823
Tympanoplasty	1	(14.3)	3	(4.4)	3.61	0.323–40.316	0.297
Ophthalmologic disorder***	1	(14.3)	19	(27.9)	0.43	0.048–3.811	0.448
Mental health							
Depression	1	(14.3)	7	(10.3)	1.45	0.152–13.875	0.746
Suicide	0	(0.0)	1	(1.5)	n/a		
Epilepsy	0	(0.0)	3	(4.4)	n/a		
Kidney diseases							
Kidney stone	0	(0.0)	1	(1.5)	n/a		
Hydronephrosis	0	(0.0)	4	(5.9)	n/a		
Hypoplastic kidney	0	(0.0)	2	(2.9)	n/a		
Horseshoe kidney	0	(0.0)	2	(2.9)	n/a		
Pyelectasis	0	(0.0)	2	(2.9)	n/a		

Notes: *Streak gonad, hypoplastic uterus, atrophic endometrium, **Widely spaced nipples, underdeveloped breasts, ***Strabism, astigmatism, hypermetropy. Bold indicates significant results ($p<0.05$) or statistically non-significant trends ($0.05<p<0.10$).
Abbreviation: US, ultrasound.

analysed features and comorbidities, we also dichotomized and analysed the cohort accordingly. Statistical analysis was performed using the IBM SPSS Statistics for Windows Version 25.0 software (IBM Corp., Armonk, NY). For continuous variables, Kolmogorov-Smirnov test was used to check normality of distribution and Levene's test was used to determine equality of variances. For parametric variables, independent sample *t*-test was used to compare equality of means, and Pearson's correlation to determine the degree of association. For non-parametric variables Spearman correlation for association and Mann–Whitney *U*-test for comparison of means were used. Nominal variables were compared using the Chi-square test or Fisher's exact test. Logistic regression was used to determine odds ratios and strength of association for binary outcomes. A p value < 0.05 was considered statistically significant. Data are shown as mean ± SD, median, or odds ratio (OR) and 95% confidence interval (CI), where applicable.

Table 5 Baseline Characteristics, Physical Features and Comorbidities of the Subgroups Diagnosed Before (<12 years of Age) and After (≥12 years of Age) the Average Age of Menarche

Baseline characteristics	**<12y at diagnosis (n=44)**		**>12y at diagnosis (n=31)**				
	Mean	**(SD)**	**Mean**	**(SD)**	**p**		
Age at diagnosis (y)	5.5	3.3	15.3	3.0	**0.000**		
Height at diagnosis (cm)	115.8	20.7	143.0	12.0	**0.000**		
Weight at diagnosis (kg)	33.6	19.2	52.3	15.8	**0.046**		
BMI at diagnosis (kg/m2)	23.4	6.4	26.6	8.8	**0.066**		
	n	**(%)**	**n**	**(%)**	**OR**	**(95% CI)**	**p**
Growth disorder							
Short stature	40	(90.9)	22	(71.0)	**4.09**	**1.129–14.825**	**0.03**
GH therapy	39	(88.6)	19	(61.3)	**4.93**	**1.516–16.010**	**0.008**
Turner phenotype							
Breast abnormality**	34	(77.3)	13	(41.9)	**4.71**	**1.727–12.835**	**0.002**
Hypertrichosis	7	(15.9)	6	(19.4)	0.79	0.237–2.624	0.998
Low hairline, flat face	31	(70.5)	13	(41.9)	**3.30**	**1.260–8.653**	**0.015**
Pterygium colli	11	(25.0)	6	(19.4)	1.39	0.452–4.266	0.566
Low set, prominent ears	27	(61.4)	12	(38.7)	**2.52**	**0.979–6.461**	**0.055**
Striae	8	(18.2)	6	(19.4)	0.93	0.286–2.998	0.898
Acanthosis or naevi	10	(22.7)	9	(29.0)	0.72	0.252–2.051	0.537
Gynecology							
Hypoplastic vagina	2	(4.5)	2	(6.5)	0.69	0.092–5.186	0.719
Abnormal pelvic US*	20	(45.5)	17	(54.8)	0.69	0.273–1.728	0.424
Clitoromegaly	2	(4.5)	2	(6.5)	0.69	0.092–5.186	0.719
Cardiac							
Hypertension	1	(2.3)	6	(19.4)	**0.10**	**0.011–0.852**	**0.035**
Tachycardia	4	(9.1)	6	(19.4)	0.42	0.107–1.624	0.207
Aortic stenosis	3	(6.8)	1	(3.2)	2.20	0.218–22.151	0.505
Mitral valve prolapse	8	(18.2)	1	(3.2)	**6.67**	**0.789–56.356**	**0.082**
Tricuspid valve insufficiency	0	(0.0)	1	(3.2)	n/a		
Atrial septum defect	5	(11.4)	4	(12.9)	0.87	0.213–3.521	0.84
Coarctation of the aorta	2	(4.5)	1	(3.2)	1.43	0.124–16.485	0.775
Patent ductus arteriosus	3	(6.8)	0	(0.0)	n/a		
Aortic insufficiency	3	(6.8)	1	(3.2)	2.20	0.218–22.151	0.505

(*Continued*)

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Table 5 (Continued).

Endocrinology, metabolism							
Obesity	9	(20.5)	8	(25.8)	0.74	0.249–2.194	0.586
Type 1 diabetes mellitus	3	(6.8)	3	(9.7)	0.68	0.128–3.631	0.655
Hypothyroidism	20	(45.5)	16	(51.6)	0.78	0.311–1.962	0.599
Hyperthyroidism	1	(2.3)	1	(3.2)	0.70	0.042–11.597	0.802
Hashimoto thyroiditis	7	(15.9)	7	(22.6)	0.65	0.202–2.083	0.467
Gastroenterology							
Celiac disease	1	(2.3)	3	(9.7)	0.22	0.021–2.193	0.195
Ulcerative colitis	0	(0.0)	3	(9.7)	n/a		
Hepatosplenomegaly	2	(4.5)	10	(32.3)	**0.10**	**0.02–0.498**	**0.005**
Sensory functions							
Anosmia	1	(2.3)	0	(0.0)	n/a		
Hypacusis	6	(13.6)	7	(22.6)	0.54	0.162–1.805	0.318
Tympanoplasty	2	(4.5)	2	(6.5)	0.69	0.092–5.186	0.719
Ophthalmologic disorder***	12	(27.3)	8	(25.8)	1.08	0.380–3.059	0.888
Mental health							
Depression	3	(6.8)	5	(16.1)	0.38	0.084–1.728	0.211
Suicide	0	(0.0)	1	(3.2)	n/a		
Epilepsy	3	(6.8)	0	(0.0)	n/a		
Kidney diseases							
Kidney stone	1	(2.3)	0	(0.0)	n/a		
Hydronephrosis	2	(4.5)	2	(6.5)	0.69	0.092–5.186	0.719
Hypoplastic kidney	1	(2.3)	1	(3.2)	0.70	0.042–11.597	0.802
Horseshoe kidney	1	(2.3)	1	(3.2)	0.70	0.042–11.597	0.802
Pyelectasis	1	(2.3)	1	(3.2)	0.70	0.042–11.597	0.802

Notes: *Streak gonad, hypoplastic uterus, atrophic endometrium, **Widely spaced nipples, underdeveloped breasts, ***Strabism, astigmatism, hypermetropy. Bold indicates significant results ($p<0.05$) or statistically non-significant trends ($0.05<p<0.10$).
Abbreviation: US, ultrasound.

Results

Baseline Characteristics

We enrolled 75 patients diagnosed with TS confirmed by karyotyping. Most patients were diagnosed with TS during childhood or adolescence (Figure 1A): 44 (58%) were diagnosed below the age of 12 years, 26 (35%) between 12 and 18 years, and only 5 (7%) at >18 years. The average age at diagnosis was 9.6 years. The baseline characteristics of the cohort are presented in Table 1. Although the age at diagnosis could be anything between 0 and 22 years, the 2 peaks with 8–8 patients (10.7–10.7%) diagnosed at that specific age were soon after birth at the age of 1, and at the time of the expected first menstrual period at 12 (Figure 1B). As the manifestation of several examined comorbidities is obviously dependent on the length of follow-up and the age at which follow-up ends (for example hypertension, obesity, thyroid disease), it is worthy of note and the results are to be

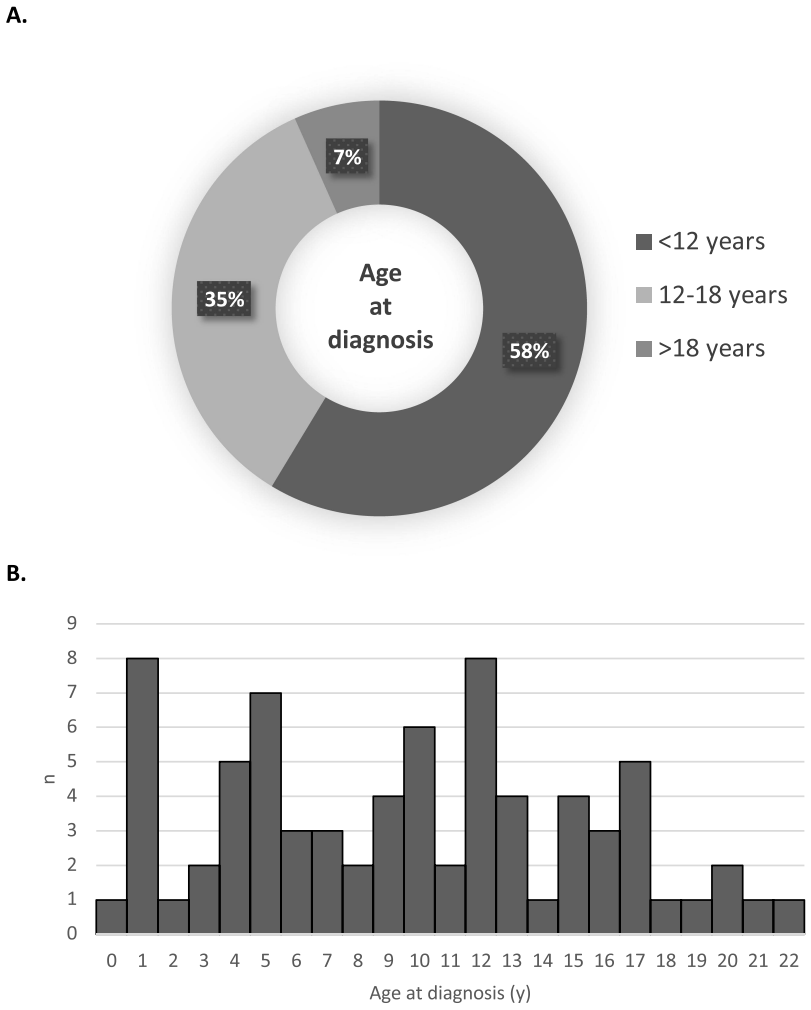

Figure 1 Age at diagnosis of Turner syndrome. (**A**) Distribution of childhood, adolescence, and adulthood diagnosis. (**B**) Frequencies of age at diagnosis at specific ages (0–22 years).

examined in the light of the fact that in our cohort the median age at diagnosis was 10 years, the median age at the time of the study was 24 years and the median follow-up time was 14 years.

Karyotype Distribution

The cytogenetic testing of the 75 cases revealed 13 different karyotypes. 47/75 (62.7%) were one of the 6 non-mosaic forms, whereas 28/75 (37.3%) were one of the 7 different mosaic karyotypes. By far the most common karyotype was the classic form, either 45,X monosomy, or mosaicism with 45,X/46,XX karyotype. Besides the rare variations, 7–7 cases (9.3–9.3%) had either the Xq isochromosome [i(Xq)] or the X ring chromosome [r(X)] present. Altogether, 66.7% had numeric, 13.3% purely structural, and 20% combined numeric and structural abnormalities (Table 6).

Mosaic vs Non-Mosaic Karyotypes

Although they just missed statistical significance with p being 0.055 and 0.06, the presence of short stature (89% vs 71%, OR 0.30) and pterygium colli (ie, webbed neck) (29% vs 10%, OR 0.28) were more common in the non-mosaic form, and hypertrichosis was also more common, actually only present in the non-mosaic group (27.7% vs 0%). In the mosaic group, however, mitral valve prolapse was diagnosed 4-times more frequently (21.4% vs 6.4%, OR: 4.0). Interestingly, when TS with mosaicism (45,X/46,XX) (n = 12) was compared with the pooled data of all the other karyotypes,

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Table 6 Distribution of Karyotypes in the Study Cohort

	Karyotype	n	(%)
All karyotypes		75	(100)
Non-mosaic		47	(62.7)
Mosaic		28	(37.3)
Numeric		50	(66.7)
Non-mosaic	45,X	35	(46.7)
Mosaic	45,X/46,XX	9	(12.0)
	45,X/46,XY	3	(4.0)
	45,X/47,XXX	3	(4.0)
Structural		10	(13.3)
Non-mosaic	46,X,i(Xq)	6	(8.0)
	46,X,del(Xp)	2	(2.7)
	46,XX,del(X)q21	1	(1.3)
Mosaic	46,X,i(Xq)/47,XX,i(Xq)	1	(1.3)
Combined (numeric/structural)		15	(20.0)
Non-mosaic	45,X,inv(10)	2	(2.7)
	45,X,inv(9)(15)	1	(1.3)
Mosaic	45,X/46,X,r(X)	7	(9.3)
	45,X/46,X,+mar	3	(4.0)
	45,X/46,X,del(X)	2	(2.7)

hypertension was associated with this karyotype (3/9 cases, 33.3% vs 4/66 cases, 6.1%, OR 7.75, 95% CI 1.39–43.08, p = 0.019; not shown in table) (Table 1).

45,X vs Other Karyotypes

When compared with other karyotypes, the classic karyotype with 45,X monosomy was found to be associated significantly or nearly significantly with short stature (91% vs 75%, OR 3.56, 95% CI 0.89–14.17, p = 0.072), hypertrichosis (28% vs 7%, OR 4.9, 95% CI 1.23–19.72, p = 0.02) and pterygium colli (34% vs 12%, OR 3.65, 59% CI 1.13–11.74, p = 0.03), and abnormal pelvic ultrasonogram with streak gonads (60% vs 40%, OR 2.25 59% CI 0.89–5.68, p = 0.086). There was no significant difference in the occurrence of comorbidities between the two groups. Probably not relevant clinically, but statistically significant difference could be seen in the age, and consequently the height and weight of the two groups, with karyotype with 45,X group being diagnosed on average 0.7 years later (Table 2).

Role of Xq Isochromosome

When compared with karyotypes that do not contain i(Xq), the Xq isochromosome seemed to be associated with celiac disease (28% vs 3%, OR 13.2, 95% CI 1.5–114.5, p = 0.019). When, however, we compared the Xq isochromosome containing karyotype to the classic karyotype with 45,X monosomy, to see if the excess Xq arm material could play a role in the differences in phenotype, no significant association was found (Table 3).

Role of Ring(X) Chromosome

In our cohort, no difference between the subgroup that contains r(X) chromosome, and the r(X) free group could be identified in the examined parameters. In the ring(X) chromosome group, short stature was less common (57% vs 85%), but the magnitude of association just missed statistical significance (OR 0.23, 95% CI 0.045–1.186, p = 0.079) (Table 4).

Age at Diagnosis

Differences in the baseline age, height and weight between the diagnosis at <12 years-of-age and the ≥12 years-of-age is self-evident. There was a nearly significant baseline difference in BMI, too, and the later-diagnosed group was more overweight (23.4 vs 26.2 kg/m2, p = 0.066). In the group that was diagnosed at a younger age, the typical TS features that were more common were short stature (91% vs 71%, OR 4.09) and GH therapy (88% vs 61%, OR 4.93), underdeveloped breasts and widely spaced nipples (77% vs 42%, OR 4.71), low hairline or dysmorphic face (71% vs 42%, OR 3.30), and low-set, prominent ears (64% vs 39%, OR 2.52). Hypertension was less common in the earlier-diagnosed group (2% vs 19%, OR 0.10), whereas mitral valve prolapse was more frequently reported (18% vs 3%, OR 6.67). It is noteworthy that hepatosplenomegaly was much less common in the earlier-diagnosed group (4% vs 32%, OR 0.10), and that all three epileptic cases were among those that were diagnosed below the age of 12 (7% vs 0%) (Table 5).

Discussion

Turner syndrome (TS) is one of the most common non-heritable genetic disorders compatible with life. It is caused by the partial or complete loss of the second sex chromosome. It might affect mental health and cognition, the development of the cardiovascular system, growth, and metabolism, which all contribute to the occurrence of typical features and complications of TS. The *SHOX* gene located on the pseudoautosomal region of the X chromosome at Xp22.3 has been considered to be one of the genes whose alteration may be associated with the signs of TS, specifically short stature.[9] The relevance of hypomethylation has also been emphasized.[14,15] Using gene mapping individual genes have been identified which might be connected to the Turner syndrome associated complications like aortic aneurysm and dilation (zinc finger FYVE-type containing 9, ZFYVE9 gene), obesity (cannabinoid receptor 1, CNR1 gene), and the insulin-like-growth factor system (insulin like growth factor binding protein 3, IGFBP3 gene).[15] Furthermore, distinction needs to be made between non-mosaic and mosaic TS patients. The genetic alterations which cause the specific phenotype of TS are expressed only in a certain percentage of cells in mosaic TS patients inducing decreased penetrance of specific features and comorbidities.[16,17] Despite all this knowledge, the exact genetic background TS features and comorbidities is either not known, or conflicting because different studies have yielded ambiguous results. In our study, we attempted to point to some correlations between the specific genetic backgrounds and symptoms.

The distribution of the age at TS diagnosis that we found is similar to the findings of other groups, regarding both the ratios of childhood, adolescent and adult age diagnosis, and the peaks of age at diagnosis. The latter occurs at 1 year's of age and around the average age of menarche, usually around 11–12 years.[7,18] This can easily be explained by the fact that either prominent physical features or the socially strictly checked and awaited beginning of the reproductive period marked by the first menstruation can be the triggers of medical examinations.

The prevalence of the TS-associated features and comorbidities in our whole cohort were higher than in the general population, as it had been expected (Table 1). Yet, this is not surprising, since the variables we chose for analysis are the well-established TS-specific features and characteristics.[7,8] Also, the distribution of the karyotypes (Table 6) are also in line with former reports about larger cohorts: the classic karyotype with 45,X monosomy was the most common; two thirds of the cases were numerical abnormalities, and more than one-third were mosaic.[6–8,18,19]

In our study we found clear differences according to the age at diagnosis: in those diagnosed earlier, physical features were more pronounced, which probably was the reason why these girls were diagnosed at a younger age. The earlier Turner syndrome is diagnosed, the more optimal the medical care of patients can be, thus improving the clinical outcomes and the patients' quality of life.[13,20] Suboptimal care provided to affected girls and adult women has been shown to lead to increased morbidity and mortality,[21] although TS in itself already results in increased mortality.[18,19] However, it is worth mentioning that when we examined the characteristics of specific karyotypes (mosaicism in general; 45,X; i(Xq); r(X)), none of these was associated with early diagnosis, that is, at an age younger than 12 years. This

suggests that phenotypical features are more related to early diagnosis than a certain karyotype. This said, further questions arise if we look at the comorbidity associations according to the age at diagnosis: although the presence of most diseases did not seem to depend on the age at TS diagnosis, some proved to be associated. Hypertension was present in only 2% of the early-diagnosis group, as compared to 20% of the late-diagnosis patients. One could explain this by the fact that hypertension develops over time, and at the end of the follow-up period of our study, the early-diagnosis group was significantly younger than the late-diagnosis group (20.1±7.1 vs 32.2±9.2 years). Nevertheless, both groups are very young for such high rates of hypertension, and this certainly can be attributed to TS.[18,19] The other two differences that we saw in comorbidities, however, can be explained less readily: mitral valve prolapse was common in the early-diagnosis group (18% vs 3%), whereas hepatosplenomegaly was frequent in the late-diagnosed group (32% vs 4%). Mitral valve prolapse, along with atrial septum defect, were the two most common structural cardiac anomalies in our cohort, both affecting 12% of the patients. The association of mitral valve prolapse with early diagnosis might be due to the clinical symptoms that occur in this group. The question remains, though, why exactly mitral valve prolapse is associated with the early diagnosis of TS and why other cardiac malformations are not. As far as the increased prevalence of hepatosplenomegaly in the late-diagnosis group is concerned, we can speculate that transient or permanent estrogen deficiency for a longer period of time might play a role. Impaired liver function and a fivefold chance for cirrhosis have been reported in patients with TS.[22,23] Our data showed higher prevalence of hepatosplenomegaly (16% of the whole cohort), in most cases in the form of hepatic steatosis. For comparison: hepatic disorders are present in only about 1–2% of the general adult population of the US.[24] It is well known that excess weight can cause liver steatosis Interestingly, and obesity is more common in TS patients (22% in our cohort), yet no relationship between obesity and increased liver enzymes or steatosis have been found in TS patients. Rather, it is supposed to originate from minimal abnormalities leading to nodular architectural changes.[22] Cirrhosis, nodular regenerative hyperplasia (NRH) and multiple focal nodular hyperplasia (FNH) were observed in TS patients as definitive structural changes. Recent studies demonstrated the positive effects of estrogen in TS: it reduces liver fat storage, and blocks insulin signaling in the liver.[23] It has been concluded that in TS-related liver changes estrogen replacement therapy may even improve the liver function. Thus, delayed diagnosis and the resulting postponed estrogen treatment in the late-diagnosis (>12 years-of-age) group may well contribute to the significantly more frequent occurrence of hepatomegaly.

Comparing the non-mosaic form of TS with mosaic patients, some characteristics (short stature, pterygium colli, hypertrichosis) were significantly more frequently reported in the non-mosaic group (Table 1). It is not surprising, as we can expect to see the typical TS phenotype if a higher percentage of the cells have the classic 45,X karyotype. This was also supported by a separate analysis of the classic non-mosaic 45,X karyotype (Table 2). However, among the analysed concomitant diseases none seemed to be more frequent in mosaicism than in non-mosaic karyotypes, or in the 45,X than in the pooled "other" group. Interestingly, when the 9 cases with 45,X/46,XX mosaicism were compared to the rest of the study cohort, increased risk of hypertension was found (OR 7.75; nor shown in table). Although detailed analysis of the various karyotypes was limited by the small number of cases in the less frequent karyotype subgroups, Xq isochromosome (Table 3) and ring(X) chromosome (Table 4) containing karyotypes could be compared with those that did not have these rearranged chromosomal structures. The only specific difference that we found was the common appearance of the i(Xq) chromosome in celiac disease (28% vs 3% in the non-i(Xq) group, OR: 13.2). As the Xq and Xp arm gene dosage seems to be crucial in some karyotype-disease associations,[6] we wanted to see if the decreased dosage of Xq genes in classic karyotype with 45,X patients and the increased presence of Xq genes in 46, Xi (Xq) result in any difference in comorbidities in general and in celiac disease in particular (Table 3), but this direct comparison yielded no significant association.

Determining direct correlation between one specific TS karyotype and one or more phenotypical feature or comorbidity has been attempted by several groups.[6–8,18,19,25–29] As rare karyotypes are present in relatively small numbers even in large nationwide analyses, it is difficult to draw epidemiologic conclusions that are karyotype specific. It is important to emphasize that, for statistical reasons, rare karyotypes are often pooled (like "mosaic", "isochromosome Xq containing") just as we also did. This, however, can also be useful, because finding an association with those pooled data can be used in clinical risk-assessment if we can place our patient in one of those pools. Unfortunately, these results are often difficult to compare and are inevitably conflicting because the exact karyotype compositions of these pools are slightly different. Another difficulty of the comparison of results might originate from different comorbidity prevalence in

different geographic regions, as well as national screening strategies for these comorbidities in TS patients. Nevertheless, some genotype-phenotype associations proposed by other groups are the following: mosaicism with 45,X/46,XX and 45, X/46,X,i(Xq) may be associated with hypothyroidism.[29] 45,X and Xq isochromosome are associated with increased mortality;[18] 45,X/46,XX seemed to be associated with the least comorbidities, and i(Xq) with cardiovascular protection (less bicuspid valves, decreased aortic size index), less hearing loss and less thyroid autoimmune diseases.[8] Mosaicism with 45,X/46,XY showed association with less height deficit, less hearing loss, less hypothyroidism[8] ring(X) was found to be associated with metabolic syndrome (increased HbA1c, GGT, hypertension) and increased height deficit,[8] and i(Xq) with increased diabetes mellitus risk as compared to 45,X monosomy.[6] In light of these literature data, our results detailed above raise the possibility of some novel associations: we found non-mosaic karyotypes and specifically the karyotype with 45,X monosomy associated with short stature, hypertrichosis and pterygium colli, 45,X/46,XX with hypertension, and the presence of the isochromosome Xq may be associated with the increased risk of celiac disease.

The major strength and limitation of this study have the same origin. The single-center characteristic of the study is on the one hand a strength of the study since patient follow-up is easier and diagnostic and follow-up strategies are uniform for all patients. However, the greatest weakness of the study has the same origin: the size of the cohort is not big enough to carry out meaningful statistical analysis in all subgroups that have small number of cases. Our results therefore warrant further confirmation in multicentric research and in bigger cohorts.

Conclusion

In this study, we have managed to determine the descriptive characteristics of a Hungarian Turner syndrome cohort of 75 patients (age at diagnosis, frequencies of typical TS physical features and comorbidities). We determined some physical characteristics and comorbidities that seem to be associated with certain specific karyotypes (mosaicism vs non-mosaic karyotype; 45,X monosomy vs other karyotypes; Xq isochromosome present vs absent). When we compared groups diagnosed before or after 12 years of age, we found that earlier diagnosis was associated with a higher odd for short stature, GH therapy, breast abnormality, dysmorphic face and mitral valve prolapse, while in those diagnosed at a later age hypertension and hepatosplenomegaly were more common. These findings can make possible targeted screening and for some features and comorbidities depending on the exact karyotype and the age of diagnosis of TS.

Abbreviations

Anti-TPO, Anti-thyroperoxidase autoantibody; anti-tTG, anti-tissue transglutaminase; BMI, Body mass index; CNR1 GENE, Cannabinoid Receptor 1 gene; FNH, Focal nodular hyperplasia; GGT, Gamma-glutamyl transferase; HBA1C, Glycated haemoglobin; IGFBP3, Insulin-like growth factor-binding protein 3; IVF, In vitro fertilization; NRH, Nodular regenerative hyperplasia; rGH, recombinant growth hormone; SHOX (gene), Short stature homeobox (gene); TS, Turner syndrome; ZFYVE9 (gene), Zinc Finger FYVE-Type Containing 9 (gene).

Data Sharing Statement

The authors confirm that the data supporting the findings of this study are available within the article.

Ethical Approval

DE RKEB/IKEB 5953-2022-2022.01.19. (University of Debrecen, Regional and Institutional Ethics Committee). This research complies with the Declaration of Helsinki.

Consent for Publication

We confirm that the patients were enrolled in the study in adult age, and the consent of cases for publication has been obtained.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors report there are no competing interests to declare.

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