Rare manifestations and malignancies in tuberous sclerosis complex: Findings from the TuberOus SClerosis registry to increAse disease awareness (TOSCA)

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Abstract

Background

Tuberous sclerosis complex (TSC) is a rare multisystem autosomal dominant genetic disorder caused by pathogenic mutations in either the TSC1 or TSC2 gene. Common manifestations of TSC have been grouped into major and minor clinical diagnostic criteria and assessed in clinical routine workup. However, case studies point towards the existence of rare disease manifestations and to the potential association of TSC with malignant tumors. In this study we sought to characterize rare manifestations and malignancies using a large cohort of patients.

Methods

Tuberous Sclerosis registry to increase disease awareness (TOSCA) is a multicenter, international disease registry collecting clinical manifestations and characteristics of patients with TSC, both retrospectively and prospectively. We report rates and characteristics of rare manifestations and malignancies in patients with TSC who had enrolled in the TOSCA registry. We also examined these manifestations by age, sex, and genotype (TSC1 or TSC2).

Results

Overall, 2211 patients with TSC were enrolled in the study. Rare manifestations were reported in 382 (17.3%) study participants and malignancies in 65 (2.9%). Of these rare manifestations, the most frequent were bone sclerotic foci (39.5%), scoliosis (23%), thyroid adenoma (5.5%), adrenal angiomyolipoma (4.5%), hemihypertrophy and pancreatic neuroendocrine tumors (pNET; both 3.1%). These rare manifestations were more commonly observed in adults than children (66.23% vs. 22.7%), in females versus males (58.4% vs. 41.6%; except for scoliosis: 48.9% vs. 51.1%), and in those with TSC2 versus TSC1 (67.0% vs. 21.1%; except for thyroid adenoma: 42.9% vs. 57.1%). In the 65 individuals with reported malignancies, the most common were renal cell carcinoma (47.7%), followed by breast (10.8%) and thyroid cancer (9.2%). Although malignancies were more common in adult patients, 26.1% were reported in children and 63.1% in individuals < 40 years. TSC1 mutations were over-represented in individuals with malignancies compared to the overall TOSCA cohort (32.1% vs. 18.5%).

Conclusion

Rare manifestations collectively were observed in a significant proportion of individuals with TSC. We recommend further examination of rare manifestations in TSC. Collectively, malignancies were infrequent findings in our cohort. However, compared to the general population, malignant tumors occurred earlier in age and some tumor types were more common.

Introduction

Tuberous sclerosis complex (TSC) is a rare autosomal dominant genetic disorder caused by pathogenic mutations in the TSC1 or TSC2 gene. This results in hyperactivation of the mammalian/mechanistic target of rapamycin pathway, leading to hamartoma formation. The prevalence of TSC is estimated to be between 1/6800 and 1/15,000 and the incidence is estimated to be nearly 1:6000–10,000 live births (1–3). It can affect all organ systems, leading to diverse clinical manifestations and has a broad variability, not only among individual patients but also within the affected families (1).

The 2012 International Tuberous Sclerosis Complex Consensus Conference provided recommendations to standardize the approach to manage this disorder. A system of major and minor criteria serves as a basis to establish the diagnosis clinically. Recommendations on surveillance and treatment mainly focus on these criteria (4). However, the involvement of multiple organ systems at different stages of life presents a major challenge in the comprehensive clinical management of patients with TSC.

Over the last two decades, several clinical pathologies have been identified as associated with TSC, such as aortic and intracranial aneurysm (5, 6), arachnoid cysts (7, 8), lymphedema (9, 10), pancreatic endocrine tumor (11), pituitary adenoma (7), chordoma (12), and bone sclerotic foci (13). Although these clinical pathologies are less frequent and not included as clinical diagnostic criteria, some of them can be life-threatening, while others may be challenging to integrate into a comprehensive clinical picture of the patients. Identification of these rare or less frequent manifestations and their clinical characteristics may help in their early diagnosis and contribute to ultimately preventing morbidity and mortality in patients with TSC.
A second important controversy in TSC is the fact that it remains a debate whether patients with TSC have an increased risk for malignant tumors. Until now, mostly renal cell carcinoma (14) has been identified in this context.

To the best of our knowledge, no systematic evaluation of these manifestations has been performed to date in a large patient cohort that might provide reliable results to aid clinicians in the management of TSC. Our aim is to present the rates and characteristics of various rare manifestations and malignancies observed in patients with TSC enrolled in the TuberOus SClerosis registry to increase disease awareness (TOSCA) study and report differences in these rare manifestations and malignancies by sex, age, and TSC mutation.

**Materials And Methods**

**Study design, participants and data collection**

A detailed methodology of the study has been published previously (15). In brief, TOSCA is a multicenter, international disease registry structured to collect patient and disease information retrospectively and prospectively. It consists of a 'core' section and six 'petals' or 'research projects'. In the core section of the study, information on the patient's background, including demographics, familial and prenatal history, vital signs, and disease features, were collected at baseline and updated annually, wherever possible. Additional detailed information was collected in the six research projects that focused on subependymal giant cell astrocytoma, renal angiomyolipoma and lymphangiomatosis, genetics, TSC-associated neuropsychiatric disorders (TAND), epilepsy, and quality of life. Here, we present data on the rare manifestations, comorbidities and malignancies from the core section of the TOSCA registry.

Patients of any age with a documented visit for TSC in the preceding 12 months or newly diagnosed with TSC were enrolled into the TOSCA registry after obtaining written informed consent. Investigators collected data on rare manifestations by either selecting predefined items known to be associated with TSC or entering other items in a free text field.

For malignancies, data were collected for those patients who had either one or more of a number of predefined malignancies (renal, ovarian, testicular, and gastrointestinal malignancies). In addition, investigators could enter any other malignancy in a free text field.

**Assessments**

Demographic and baseline characteristics between the patients with and without rare manifestations, and between patients with and without malignancies, were compared. For the purpose of this study, we defined rare manifestations as all manifestations declared as rare and disease-related by the investigators that did not fit under major or minor TSC clinical criteria as outlined in the 2012 Tuberous Sclerosis Consensus Conference recommendations and that were not a clear sequelae of major or minor manifestations (such as epilepsy).

We have prospectively categorized the rare manifestations by sex and mutation type, and by several syndromal complexes, such as tumors, malformations, vascular malformations, cystic lesions, endocrine disorders, and others (Table 2). Rare manifestations were also categorized into the following organ classes: vasculature, ear, nose and throat, endocrine system, eye, heart, gastrointestinal, liver, lymphatic tissue, nervous system, skeletal, urogenital, and others (Supplementary Table 1).

Rare manifestations reported by investigators in the free text field of the case report form that did not have an unambiguous description, were clearly not rare manifestations, or were typical manifestations of TSC (major/minor diagnostic criteria or epilepsy) were excluded from the analysis (Supplementary Table 2).

Malignancies observed in the TOSCA cohort were reported by organ. Rare manifestations and malignancies (either predefined or open field) were grouped based on organ system, and further by age, sex, and genotype. Available information on the number of patients with rare manifestations and malignancies who received treatment is reported.

**Statistical analysis**

All patients, without any major protocol deviation, enrolled in the TOSCA clinical study were included for analysis. SAS® Version 9.2 or later was used to perform all statistical analyses. Continuous variables were summarized with descriptive statistics (n, mean, standard deviation, range [minimum and maximum] and median). Frequency counts and the percentage of patients within each category were used for categorical data. Demographic and baseline characteristics between patients with and without rare manifestations and between patients with and without malignancies were compared using chi-square test for association and Z-test for means, as appropriate. A p-value <0.05 was considered statistically significant.

**Results**
Patient demographics and clinical characteristics

A total of 2211 patients with TSC were enrolled in the TOSCA registry. Of those, 382 (17.3%) individuals had rare manifestations and 65 (2.9%) had malignancies reported by investigators. Demographic characteristics in patients with and without rare manifestations and malignancies are shown in Table 1.

In patients with rare manifestations, TSC was diagnosed later than in those without (median: 2 years vs. 1 year; p<0.0001). The female to male ratio was higher in patients with rare manifestations than in those without (female vs. male; 58.4% vs. 41.6% compared to 50.8% vs. 49.2%, respectively; p=0.007). There was no difference in the mutation types (TSC1, TSC2 or no mutation identified [NMI]) in participants with rare manifestations compared to individuals without (p=0.687).

In patients with malignancies, TSC was diagnosed later compared to those without malignancies (median: 10.5 years vs. 1 year; p<0.0001). We found a higher female to male ratio in patients with malignancies compared to individuals without malignancy (66.2%:33.8% vs. 51.7%:48.3%; p=0.021). Considering differences in mutations, participants with malignancies had a significantly higher rate of TSC1 mutations than participants without malignancies (TSC1 vs. TSC2 vs. NMI; 32.3% vs. 48.4% vs. 19.4% and 18.5% vs. 64.7% vs.14.5%, respectively; p=0.037).

Rare manifestations

In this study, 88 different manifestations were identified that were designated by the investigators as rare TSC-associated manifestations (Table 2). The five most frequent rare manifestations were bone sclerotic foci (39.5%), scoliosis (23%), thyroid adenoma (5.5%), adrenal angiomyolipoma (4.5%), hemihypertrophy and pNET (3.1% each) (Table 3).

Of the 382 patients with rare manifestations, tumors and cystic lesions were reported in 268 (tumors in 248 patients and cystic lesions in 20 patients), with female patients being more commonly affected than males (60.5% vs. 39.5%). Malformations were reported in 124 patients, equally affecting both sexes (male vs. female; 51.3% vs. 48.7%) and a majority of patients having TSC2 mutation (85.2%). Malformations were observed at an earlier age (median age, 11 years), while tumors (median age, 28 years), cystic lesions (median age, 27 years), and endocrine disorders (median age, 31.5 years) were observed at a later age (Table 4).

As reported above, rare manifestations were predominant in female compared to male patients, except for scoliosis which was a little more frequent in male patients (51.1% vs. 48.9%; p=0.733). Similar distribution patterns of TSC gene mutation were noted in patients with or without rare manifestation, i.e. rate of TSC2 mutation being more than TSC1, with the exception of patients with thyroid adenoma who had a higher rate of TSC1 mutations than TSC2 (57.1% vs. 42.9%; p=0.512). Upon stratification by age group, rare manifestations were more common in adult patients (>18 years) (Table 5).

Malignancies

Malignancies were reported in 65 patients. Most frequent malignancies observed were renal cell carcinoma (47.7%), breast cancer (10.8%), and thyroid cancer (9.2%).

Altogether, the percentage of female patients was significantly higher in the group of participants with malignancies (66.2%) compared to participants without malignancies (51.7%; p=0.021) (Table 1). The predominance of females was consistent in patients with renal cell carcinoma (64.5%), but didn’t reach statistical significance. In addition, the TSC1:TSC2 ratio was significantly higher in patients with malignancies (TSC1 vs. TSC2 vs. NMI was 32.3% vs. 48.2% vs. 19.4%) compared to individuals without malignancies (18.5%:64.7%:14.5%; p=0.037). Accordingly, the TSC1:TSC2 ratio was markedly higher (TSC1 36.4% vs. TSC2 53.3%) in patients with renal malignancy than in the patients without malignancy (p=0.037). Thyroid carcinoma (n=6) was exclusively reported in six female patients, of which half had TSC1 mutations (Table 6).

Patients with malignancy appeared to be older (median 32.7 years) than those without (median 12 years; p<0.0001). Overall, malignancies were more common in adult patients (≥18 years) compared with pediatric patients (<18 years) (Table 7). However, it is important to state that nearly one-third of renal cell carcinoma cases, half of thyroid carcinomas, half of bone and soft tissue malignancies, one-fourth of ovarian malignancies, and all of the pancreatic malignancies occurred in children (≤14 years). Overall, 26% (n=17) of malignancies were detected in patients <18 years and 63.1% (n=41) in those <40 years (Table 7). Three patients (all female) had two different malignancies: one patient had ovarian and thyroid malignancies, the second patient had colon and breast malignancies, and the third patient had renal and breast malignancies. No patient died due to malignancies during the study.

Discussion
Rare manifestations

The consensus clinical TSC diagnostic criteria include commonly presented TSC manifestations and the surveillance recommendations mainly focus on these manifestations (4). However, there are numerous additional manifestations of TSC that are reported. They may occur quite frequently (for example, bone sclerotic foci), but may be systematically missed as they are not usually of clinical relevance. This large cohort study provides a clear estimate of the frequency of rare manifestations. Bone sclerotic foci might be mainly detected as a by-product of thoracic computed tomography scans, which are recommended for adult women affected by TSC only in order to screen for lymphangioleiomyomatosis (LAM) (16). This might explain the clear female predominance of this manifestation in our study. Although of minor clinical relevance, it is important to know about this rather frequent ‘rare manifestation’ as bone sclerotic foci could be misinterpreted as bone metastasis or bone secondaries, which might result in unnecessary and potentially invasive assessments (4, 17) in spite being a common benign manifestation of TSC. Expert advice should be sought in case of doubt in these patients.

In addition, bone sclerotic lesions are discussed to differentiate TSC-associated LAM from sporadic LAM (16). The consensus guidelines further recommend excluding TSC-associated manifestations, such as bone cysts, endocrinopathies, vascular aneurysms, and gastrointestinal polyps, from routine evaluation unless coupled with clinical symptoms or history due to the insufficient evidence of benefit (1).

TSC is a multisystem disorder based on defects in tumor suppressor genes. We therefore hypothesized that there might be additional rare manifestations and asked investigators to document clinical signs considered as possible TSC-associated rare manifestations.

In total, 88 different rare manifestations were recorded in 17.3% of patients in this study. This shows the complexity of the disease and highlights the limitations of systematic evaluation and treatment of rare manifestations. Most rare manifestations were more common in female patients and those with TSC2 mutations, which is in line with findings from previous literature on rare manifestations such as lymphedema and angiomyolipoma (10, 18). Tumors and cystic lesions in a broad variety of organs seem to occur (or at least are detected) at higher ages. However, clinical significance seems limited in most cases, as treatment was reported for only 16% of tumors and 5% of cysts. It is worth noting that treatment rates differed markedly depending on organ system. Details are given in Supplement Table 1.

A relevant number of rare manifestations were malformations (occurring at younger ages), of which scoliosis and hemihypertrophy were the most frequent. Diagnosis of musculoskeletal malformations can be performed easily via careful physical examination in most cases, which might contribute to detection at an earlier age. Whereas hemihypertrophy was clearly more frequent in our cohort compared to the overall population (1 in 86,000 live births), the rate of scoliosis did not differ from reports of adolescent idiopathic scoliosis (3.8% vs. 3.3%) (19, 20). This might raise the question of whether scoliosis is a (rare) manifestation of TSC or a coincidental finding. Although causality cannot be proven by a registry study, it is worth mentioning that 22.7% of the scoliosis patients in our study required treatment (compared to 0.3% with adolescent idiopathic scoliosis). This possibly points toward a higher degree of severity or an underestimation of mild cases (21). As previously reported, vascular malformations, including arterial aneurysms, can occur in patients but seem to be quite rare (n = 5 corresponding to 0.2% of the participants) (Table 5). Therefore, our data support the 2012 consensus recommendations not to perform routine evaluation given the sparse numbers. If routine imaging of the brain is performed, it seems justifiable to screen for blood vessel abnormalities as 75% of arterial aneurysms in our cohort were reported to occur in the extra- or intracranial brain-supporting vessels (4).

Conclusion

Rare manifestations occur in a relevant percentage of TSC patients. However, variability is high and further systematic evaluations are required to shape diagnostic and surveillance strategies. Malignancies affected about 2% of the participants in our study. Compared to the overall population, malignant tumors occurred earlier in age and were more common in females and participants with TSC1 mutation.

Abbreviations

LAM: lymphangioleiomyomatosis; NMI: no mutation identified; TOSCA: TuberOus SClerosis registry to increAse disease awareness; TSC: Tuberous sclerosis complex

Declarations

Acknowledgments

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Author Contributions

MS Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. SV data interpretation, drafting, reviewing, final review, and approval of the manuscript. GBdTA. Designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. PJDV Designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. EB Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. MPB Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. TC Designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. VC Designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. PC Designing the study, patient accrual, clinical care, data interpretation, drafting, reviewing, final review, and approval of the manuscript. MD Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. LD’TDesigning the study, trial management, data collection, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. JCF Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. MF Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. CH Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. SJ Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. MF Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. JCK Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. JAL Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. AM Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. RM Designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. ACJ Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. VS Designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. SS Designing the study, trial statistician, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript.
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Consent for publication

All patients were informed that the reason for enrolling in the study was to collect natural history data for scientific analysis and publication (an obligation mandated by the Good Clinical Practice guidelines) and they signed consent forms with that understanding.

Conflict of Interest

MS, EB, TC, VC, PC, GBd’A, JCK, JCF, MF, CF, CH, SJ, RN, FO’C, JQ, RT, MD, JAL, AM, SY, MPB, ACJ, PJdV, and BZ received honoraria and support for travel from Novartis. VC received personal fees for consulting, lecture fees and travel from Actelion, Bayer, Biogen Idec, Boehringer Ingelheim, Gilead, GSK, MSD, Novartis, Pfizer, Roche, Sanofi; grants from Actelion, Boehringer Ingelheim, GSK, Pfizer, Roche; personal fees for developing educational material from Boehringer Ingelheim and Roche. PJdV has been on the study steering group of the EXIST-1, 2 and 3 studies sponsored by Novartis, and co-PI on two investigator-initiated studies part-funded by Novartis. RN received grant support, paid to her institution, from Eisai and lectures fees from Nutricia, Eisai, Advicenne and GW Pharma. YT received personal fees from Novartis for lecture and for copyright of referential figures from the journals and received a grant from the Japanese government for intractable epilepsy research. SJ was partly financed by the EC Seventh Framework Programme (FP7/2007-2013; EPISTOP, grant agreement no. 602391), the Polish Ministerial funds for science (years 2013-2018) for the implementation of international co-financed project and the grant EPI MARKER of the Polish National Center for Research and Development No STRATEGMED3/306306/4/2016. JCK, PC, CH, JAL, and JQ received a research grant from Novartis. RM, LD’A and SS are employees of Novartis. VS and SV reported no conflict of interest. This study was funded by Novartis Pharma AG. All authors approved the final version of the manuscript prior to submission.

Data Availability Statement

Novartis supports the publication of scientifically rigorous analysis that is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymized patient-level data, respecting patient informed consent, contacting study sponsor authors. The protocol can be accessed through EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

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Ethics approval and consent to participate

All patients (or their legal representatives) provided written informed consent before enrolling in the TOSCA disease registry. The study was designed, implemented, and reported in accordance with the principles of Good Clinical Practice, Good Pharmacoepidemiology Practices of the International Society for Pharmacoepidemiology (ISPE 2008), the STROBE (Strengthening and Reporting of Observational Studies in Epidemiology) guidelines, and the ethical principles laid down in the Declaration of Helsinki, and all local regulations. The study protocol and all amendments were reviewed and approved (if applicable) by independent ethics committee/institutional review board for each centre: National Hospital Organization Central Ethics Committee; Gazi University Clinical Research Ethics Committee; Independent Multidisciplinary Committee on Ethical Review of Clinical Trials; Peking Union Medical College Hospital; Commissie Medische Ethiek UZ Brussel; CNIL (Commission Nationale de l'Informatique et des Libertés), CCTIRS (Comité Consultatif sur le traitement de l’information en matière de recherche dans le domaine de la santé); Comité Ético Investigación Clínica de Euskadi (CEIC-E); Consejeria de Salud y Bienestar Social, Dirección General de Calidad, Investigación, Desarrollo e Innovación, Comité Coordinador de Ética de la Investigación Biomédica de Andalucía; Research Ethics Committee of the University of Tartu (UT REC); Ethikkommission der Medizinischen Universität Graz; North Wales REC – West; Regionala Etikprövningsnämnden i Göteborg; REK – Regionale komitee voor medisinsk og helsefaglig forskningsetik; Komisja Bioetyczna przy Instytucie "Pomnik Centrum Zdrowia Dziecka"; Ethikkommission bei der Ludwig-Maximilians-Universität München; Hokkaido University Hospital Independent clinical research Institutional Ethics Committee; Medical Juntendo University Institutional Ethics Committee; National Center for Chile Health and Development of IRB; Osaka University Hospital of IRB; Ethics Committee at Moscow Institute of Pediatrics and Pediatric Surgery; Peking University First Hospital; Sanbo Brain Hospital Capital Medical University; Tianjin Children's Hospital; Childrens Hospital Of Fudan University; Zhongshan Hospital Fudan University; Fudan University Shanghai Cancer Center; The Second Affiliated Hospital of Guangzhou Medical University; The First Affiliated Hospital, Sun Yan-Sen University; The First Affiliated Hospital Of
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Tables

Table 1. Demographic characteristics in patients with and without rare manifestations and in patients with and without malignancies
| Characteristics                                      | Patients with rare manifestations, n (%) | Patients without rare manifestations, n (%) | p value   | Patients with malignancies, n (%) | Patients without malignancies, n (%) | p value   |
|-----------------------------------------------------|----------------------------------------|--------------------------------------------|-----------|-----------------------------------|-------------------------------------|-----------|
| **Age at consent, years**                            | N=382                                  | N=1829                                     |           | N=65                              | N=2146                              |           |
| Mean (SD)                                           | 28.4 (16.71)                          | 15.0 (14.15)                               | <0.0001   | 32.7 (18.86)                      | 16.9 (15.11)                        | <0.0001   |
| Median (range)                                       | 26.0 (0–71)                           | 10.0 (0–71)                                |           | 31 (0–68)                         | 12 (0–71)                           |           |
| **Age at first TSC diagnosis, years**                | n=64                                   | n=2110                                     |           | 16.9 (18.94)                      | 6.6 (11.95)                         | <0.0001   |
| Mean (SD)                                           | 12.4 (16.68)                          | 5.8 (10.87)                                | <0.0001   | 10.5 (0–67)                       | 1 (0–69)                            |           |
| Median (range)                                       | 2.0 (0–69)                            | 1.0 (0–67)                                 |           | 10.5 (0–67)                       | 1 (0–69)                            |           |
| Sex, n (%)                                           |                                        |                                            |           |                                   |                                     |           |
| Male                                                | 159 (41.6)                            | 900 (49.2)                                 | 0.007     | 22 (33.8)                        | 1036 (48.3)                         | 0.0214    |
| Female                                              | 223 (58.4)                            | 928 (50.8)                                 |           | 43 (66.2)                        | 1109 (51.7)                         |           |
| Mutation, n (%)                                      |                                        |                                            |           |                                   |                                     |           |
| TSC1                                                | 39 (21.1)                             | 152 (18.4)                                 | NS        | 10 (32.3)                         | 181 (18.5)                          | 0.0366    |
| TSC2                                                | 124 (67.0)                            | 525 (63.6)                                 |           | 15 (48.4)                         | 634 (64.7)                          |           |
| NMI                                                 | 19 (10.3)                             | 129 (15.6)                                 |           | 6 (19.4)                          | 142 (14.5)                          |           |

NMI, no mutation identified; NS, not significant; SD, standard deviation; TSC, tuberous sclerosis complex.

Table 2: Rare manifestations observed in the study and grouped by tumors, malformations, endocrine disorders, cystic lesions, and others.
| Tumors                                      | Malformations                          | Vascular malformations         | Cystic lesions | Endocrine disorders | Others                                      |
|---------------------------------------------|----------------------------------------|---------------------------------|----------------|---------------------|---------------------------------------------|
| Cartilaginous tumors of nasal septum        | Calvarium sclerosis and thickening     | Angiomatosis femoris            | Liver cyst     | Graves's disease    | Laryngomalacia                             |
| Abdominal cystic pelvic tumor               | Coloboma of iris                       | Aneurysm of anterior cerebral artery | Ovarian cyst   | Hyperthyroidism     | Leg edema                                  |
| Adrenal angiomyolipoma                      | Club foot                              | Carotid aneurysm                | Pancreatic cystic lesion | Hyperparathyroidism | Myositis ossificans                        |
| Angiomyolipoma of uterus                    | Congenital duodenal atresia            | Chyloperitoneum                 | Pancreatic cystic lesion (small) | Hypothyroidism | Pyoderma gangrenosum                       |
| Angiomyolipoma other localization           | Foot inversion                         | Lymphangioma chyloperitoneum, Perineural cyst | Hypopituitary [hypothyroidism] | Lymphedema                                |
| Bile duct adenoma                           | Anterior polar cataract                 | Lymphangioma in pelvis cavity   | Arachnoid cyst | Lymphedema          | Lymphangiosis                               |
| Bladder lipoma                              | Hemihypertrophy                        |                                 |                |                     |                                             |
| Bone sclerotic foci                         | Hemia diaphragm                        |                                 |                |                     |                                             |
| Cardiac lipoma                              | Hip dysplasia                          |                                 |                |                     |                                             |
| Cartilaginous exostosis                     | Hyperostosis                           |                                 |                |                     |                                             |
| Chordoma                                    | Hypospadias                            |                                 |                |                     |                                             |
| Chyloperitoneum                             | Anal malformation                      |                                 |                |                     |                                             |
| Lymphangioma                                | Kyphosis                               |                                 |                |                     |                                             |
| Lymphangioma in pelvis cavity              | Cerebellum angioma                     |                                 |                |                     |                                             |
| Dermoid cyst                                | Scoliosis                              |                                 |                |                     |                                             |
| Desmoplastic fibroma sinus maxillaris       | Plantar fibromatosis                   |                                 |                |                     |                                             |
| Epidermal cyst in ear canal                 | Slipped femoral epiphyses              |                                 |                |                     |                                             |
| Fibrolipoma                                 | Spina bifida occulta                   |                                 |                |                     |                                             |
| Fibrous bone disorder                       | Syringomyelia                          |                                 |                |                     |                                             |
| Fibrous hamartoma T2 spine                  |                                        |                                 |                |                     |                                             |
| Gall bladder polyp                          |                                        |                                 |                |                     |                                             |
| Hamartoma breast                            |                                        |                                 |                |                     |                                             |
| Hamartoma stomach                           |                                        |                                 |                |                     |                                             |
| Intraductal papillary mucinous neoplasm     |                                        |                                 |                |                     |                                             |
| Liver angiomyolipoma                        |                                        |                                 |                |                     |                                             |
| Liver hamartoma                             |                                        |                                 |                |                     |                                             |
| Liver hemangioma                            |                                        |                                 |                |                     |                                             |
| Lung tumor                                  |                                        |                                 |                |                     |                                             |
| Lymph nodes from the renal resection region with a pattern of angiomyolipoma | | | | |
| Neuroendocrine carcinoid tumor grade 1 in the duodenum |
|------------------------------------------------------|
| Neurofibroma                                          |
| Osteochondrome                                        |
| Ovarian tumor                                         |
| Pancreatic angiomyolipoma                             |
| Pancreatic hamartoma                                  |
| Pancreatic tumor                                      |
| Parathyroid adenoma                                   |
| Parathyroid nodule                                    |
| Part solid and part cystic lesion in pancreas tail    |
| PEComa [perivascular epithelial cell tumor] of the uterus or ovary |
| PEComa heart                                          |
| PNET                                                  |
| Polypoid lesion in gall bladder                       |
| Secreting pituitary adenoma                           |
| Spleen angiomyolipoma                                 |
| Spleen hamartoma                                      |
| Stomach hamartoma                                     |
| Struma                                                |
| Submandibular gland tumor                             |
| Thyroid adenoma                                       |
| Thyroid nodule                                        |
| Unspecified subcutaneous neoplasm in the thoracic region |
| Unspecified tumor of nasal cavity                     |
| Uterus myoma                                          |

**Table 3.** Demographics of patients with the ten most common rare manifestations by median age, sex, mutational status, and treatment
| Rare manifestations | N=2311 | Patients with rare manifestation, n (%) | Median age at diagnosis, years, n (range) | Sex, n (%) | Mutations, n (%) | Patients who received treatment, n (%) |
|---------------------|--------|----------------------------------------|------------------------------------------|------------|-----------------|-------------------------------------|
|                     |        | N=382                                  |                                          | Male (n=159) | Female (n=223) | p value | TSC1 (n=39) | TSC2 (n=124) | P value |                             |
| Bone sclerotic foci | 151 (6.5) | 151 (39.5) | 31.5 (0–70) | 59 (39.1) | 92 (60.9) | 0.0166 | 14 (24.1) | 44 (75.9) | NS | 3 (2.0) |
| Scoliosis            | 88 (3.8)  | 88 (23.0) | 13 (0–50) | 45 (51.1) | 43 (48.9) | NS | 6 (15.4) | 33 (84.6) | NS | 20 (22.7) |
| Thyroid adenoma      | 21 (0.9)  | 21 (5.5) | 38.5 (12–69) | 3 (14.3) | 18 (85.7) | 0.0015 | 4 (57.1) | 3 (42.9) | NS | 6 (28.6) |
| Adrenal angiomyolipoma | 17 (0.7) | 17 (4.5) | 22.5 (3–45) | 6 (35.3) | 11 (64.7) | NS | 0 | 3 (100) | NS | 5 (29.4) |
| pNET                | 12 (0.5)  | 12 (3.1) | 2111 (9–63) | 5 (41.7) | 7 (58.3) | NS | 2 (20.6) | 5 (71.4) | NS | 8 (66.7) |
| Hemihypertrophy      | 12 (0.5)  | 12 (3.1) | 4.5 (0–49) | 5 (41.7) | 7 (58.3) | NS | 0 | 5 (100) | NS | 1 (8.3) |
| Liver cysts          | 9 (0.4)   | 9 (2.4) | 42 (14–60) | 3 (33.3) | 6 (66.7) | NS | 1 (25.0) | 3 (75.0) | NS | 0 |
| Spleen angiomyolipoma | 9 (0.4)   | 9 (2.4) | 13 (4–60) | 4 (44.4) | 5 (55.6) | NS | 1 (20.0) | 4 (80.0) | NS | 3 (33.3) |
| Ovarian cysts        | 8 (0.3)   | 8 (2.1) | 16 (9–31) | 0 | 8 (100) | 0.0078 | 2 (33.3) | 4 (66.7) | NS | 1 (12.5) |
| Lymphedema           | 7 (0.3)   | 7 (1.8) | 21 (0–48) | 3 (42.9) | 4 (57.1) | NS | 1 (16.7) | 5 (83.3) | NS | 4 (57.1) |
| Liver angiomyolipoma | 6 (0.3)   | 6 (1.6) | 29 (16–53) | 1 (16.7) | 5 (83.3) | NS | 0 | 3 (100) | NS | 0 |

Values are expressed as n (%) unless otherwise mentioned. NS, not significant; pNET, pancreatic neuroendocrine tumor

Table 4. Rate of the ten most common rare manifestations by age group

| Age, years | n (%) | ≤2 (n=10) | 2–≤5 (n=16) | >5–≤9 (n=23) | >9–≤14 (n=49) | >14–≤18 (n=18) | >18–≤40 (n=168) | >40 (n=98) |
|------------|-------|-----------|-------------|-------------|---------------|----------------|----------------|-----------|
| Bone sclerotic foci |        | 0 | 0 | 1 (4.3) | 5 (10.2) | 5 (27.8) | 88 (52.4) | 52 (53.1) |
| Scoliosis |        | 1 (10) | 2 (12.5) | 8 (34.8) | 24 (48.9) | 6 (33.3) | 37 (22.0) | 10 (10.2) |
| Thyroid adenoma |        | 0 | 0 | 0 | 1 (2.0) | 1 (5.6) | 8 (4.8) | 11 (11.2) |
| Adrenal angiomyolipoma |        | 0 | 0 | 4 (17.4) | 2 (4.1) | 0 | 8 (4.8) | 3 (3.1) |
| pNET |        | 0 | 0 | 1 (4.3) | 3 (6.1) | 0 | 7 (4.2) | 1 (1.0) |
| Hemihypertrophy |        | 3 (30) | 1 (6.3) | 1 (4.3) | 1 (2.0) | 2 (11.1) | 3 (1.8) | 1 (1.0) |
| Liver cysts |        | 0 | 0 | 0 | 1 (2.0) | 0 | 3 (1.8) | 5 (5.1) |
| Spleen angiomyolipoma |        | 0 | 2 (12.5) | 0 | 2 (4.1) | 0 | 2 (1.2) | 3 (3.1) |
| Ovarian cysts |        | 0 | 0 | 1 (4.3) | 4 (8.2) | 0 | 3 (1.8) | 0 |
| Lymphedema |        | 1 (10) | 0 | 0 | 2 (4.1) | 1 (5.6) | 1 (0.6) | 2 (2.0) |
| Liver angiomyolipoma |        | 0 | 0 | 0 | 1 (2.0) | 0 | 3 (1.8) | 2 (2.0) |
Values are expressed as n (%) unless otherwise mentioned. pNET, pancreatic neuroendocrine tumor.

Table 5. Malformations, tumors, and other manifestations in patients with rare manifestations

| Tumors and cystic lesions, n (%) | Malformations, n (%) | Others, n (%) |
|----------------------------------|----------------------|--------------|
| **n=268**                        | **n=124**            | **n=15**     |
| **Tumors**                       | **Cystic lesions**   | **Overall**  |
| n=248                            | n=20                 | n=119        |
| **Vascular malformations**       | **n=5**              |              |
| **Others***                      |                      |              |
| **n=9**                          |                      |              |
| **Endocrine dysfunctions**       |                      |              |
| **n=6**                          |                      |              |

**Median age at diagnosis, years (range):**

- Tumors: 28 (0–69)
- Cystic lesions: 27 (10–62)
- Overall: 11 (0–50)
- Vascular malformations: 24.5 (0–50)
- Others*: 9.5 (0–48)
- Endocrine dysfunctions: 31.5 (2–48)

**Sex**

|               | Male   | Female |
|---------------|--------|--------|
| Male          | 98 (39.5) | 150 (60.5) |
| Female        | 16 (80)   | 61 (51.3)   |

**Mutation type**

|               | TSC1 | TSC2 |
|---------------|------|------|
| Male          | 27 (26.2) | 76 (73.8) |
| Female        | 3 (23.1)   | 10 (76.9)   |

**Treatment**

|               |        |        |
|---------------|--------|--------|
| Male          | 40 (16.2) | 1 (5.0)   |
| Female        | 26 (21.8) | 3 (60.0)   |

*Includes patients with laryngomalacia, lymphedema and myositis ossificans.

Table 6. Rate of malignancies in different organs in all patients, by sex and genotype

|               | Male   | Female |
|---------------|--------|--------|
| TSC1          | 46 (85.2) | 1 (100) |
| TSC2          | 3 (14.3)   | 1 (85.7)   |
| TSC1          | 0       | 6 (100)   |
| TSC2          | 0       | 3 (100)   |

**Values are expressed as n (%) unless otherwise mentioned.**
| Organs               | 5-year prevalence of malignancies in general population (per 100,000) $^5$ | All, n (%) | Sex, n (%) | Mutation, n (%) | p value |
|----------------------|------------------------------------------------------------------------------|------------|------------|-----------------|---------|
|                      | Overall                       | Male       | Female     | N=65            | Male (n=23) | Female (n=42) | TSC1 (n=11) | TSC2 (n=15) |         |
| Kidney               | 13.4                           | 16.5       | 10.3       |                 | 31 (47.7)   | 20 (47.6)    | NS          | 4 (36.4)   | 8 (53.3) | 0.3176   |
| Breast               | 181.8                          | -          | 181.8      |                 | 7 (10.8)    | 7 (16.7)     | 0.0159      | 1 (9.1)    | 1 (6.7)  | 0.3961   |
| Thyroid              | 26.2                           | 11.2       | 41.4       |                 | 0           | 6 (14.3)     | 0.0159      | 3 (27.3)   | 1 (6.7)  | 0.0104   |
| Testis               | 7.4                            | 7.4        | -          |                 | 5 (7.7)     | 0           | 0.0265      | 1 (9.1)    | 0        | 0.2230   |
| Ovary                | 20.2                           | -          | 20.2       |                 | 4 (6.2)     | NA          | NS          | 1 (9.1)    | 3 (20.0) | 1.000    |
| Bone, soft tissue    | -                              | -          | -          |                 | 2 (3.1)     | 0           | NS          | 0          | 1 (6.7)  | 1.000    |
| Colon                | 62.8                           | 67.4       | 58.0       |                 | 0           | 2 (4.8)     | NS          | 0          | 0        | NE       |
| Lung                 | 27.9                           | 34.1       | 21.6       |                 | 0           | 2 (4.8)     | NS          | 1 (9.1)    | 0        | 0.2230   |
| Pancreas             | 3.7                            | 3.9        | 3.5        |                 | 2 (3.1)     | 2 (8.7)     | NS          | 0          | 1 (6.7)  | 1.000    |
| Brain (cerebral)     | 10.1                           | 10.3       | 9.9        |                 | 1 (1.5)     | 1 (4.3)     | NS          | 0          | 0        | NE       |
| Eye                  | -                              | -          | -          |                 | 1 (1.5)     | 0           | 1 (2.4)     | NS         | 0        | 0        | NE       |
| Liver                | 8.8                            | 12.2       | 5.4        |                 | 1 (1.5)     | 1 (4.3)     | NS          | 0          | 0        | NE       |
| Skin*                | 12.7                           | 13.1       | 12.2       |                 | 1 (1.5)     | 1 (4.3)     | 0.4835      | 0          | 0        | NE       |

*Melanoma of skin. $^5$5-year prevalence of malignancies have been presented for qualitative comparison between the general population and our cohort. Values are expressed as n (%) unless otherwise mentioned. NE, non-estimable; NS, not significant

Table 7. Malignancies by organ class and age groups
| Organs       | 5-year prevalence of malignancies in general population by age group (per 100, 000) | Age (years), n (%)          |
|--------------|----------------------------------------------------------------------------------|----------------------------|
|              | 0–9 | 10–14 | 15–19 | 20–39 | >40 | ≤2 (n=1) | >2–≤5 (n=3) | >5–≤9 (n=3) | >9–≤14 (n=8) | >14–≤18 (n=2) | >18–≤40 (n=24) | >40 (n=24) |
| Kidney       | 2.1 | 0.48  | 0.51  | 1.7   | 34.3| 1 (100)  | 3 (100)     | 2 (66.7)    | 3 (37.5)     | 1 (50.0)      | 10 (41.7)       | 11 (45.8) |
| Breast       | 0.02| 1.2   | 7.7   | 46.5  | 450.5| 0         | 0           | 0           | 0           | 1 (4.2)       | 6 (25)         |
| Thyroid      | 0.28| 4.3   | 9.5   | 20.6  | 54.4| 0         | 0           | 0           | 1 (12.5)    | 1 (50.0)      | 1 (4.2)        | 3 (12.5) |
| Testis       | 0.97| 3.1   | 6.7   | 12.0  | 9.2 | 0         | 0           | 0           | 0           | 0             | 4 (16.7)        | 1 (2)    |
| Ovary        | 0.62| 2.1   | 3.9   | 9.5   | 45.7| 0         | 0           | 0           | 1 (12.5)    | 0             | 2 (16.7)        | 1 (4.2) |
| Brain        | 3.2 | 3.4   | 3.6   | 5.2   | 20.7| 0         | 0           | 0           | 1 (12.5)    | 0             | 0             | 0        |
| Colon        | 0.06| 0.82  | 1.7   | 4.7   | 170.1| 0         | 0           | 0           | 0           | 0             | 2 (8.3)        |
| Bone, soft tissue | -  | -     | -     | -     | -   | 0         | 0           | 1 (33.3)    | 0           | 0             | 0             | 1 (4.2) |
| Pancreas     | 0.01| 0.10  | 0.26  | 0.47  | 9.9 | 0         | 0           | 0           | 2 (25.0)    | 0             | 0             | 0        |
| Lung         | 0.06| 0.34  | 0.77  | 1.8   | 75.9| 0         | 0           | 0           | 0           | 1 (4.2)       | 1 (4.2)        |
| Skin*        | 0.11| 0.70  | 1.7   | 4.2   | 31.4| 0         | 0           | 0           | 0           | 0             | 1 (4.2)        | 0        |
| Eye          | -   | -     | -     | -     | -   | 0         | 0           | 0           | 0           | 0             | 1 (4.2)        | 0        |
| Liver        | 0.72| 0.49  | 0.84  | 1.8   | 22.4| 0         | 0           | 0           | 0           | 0             | 1 (4.2)        | 0        |

*Melanoma of skin. $5-year prevalence of malignancies have been presented for qualitative comparison between the general population and our cohort.