Early-Onset Osteoarthritis, Charcot-Marie-Tooth Like Neuropathy, Autoimmune Features, Multiple Arterial Aneurysms and Dissections: An Unrecognized and Life Threatening Condition

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Abstract

Background: Severe osteoarthritis and thoracic aortic aneurysms have recently been associated with mutations in the SMAD3 gene, but the full clinical spectrum is incompletely defined.

Methods: All SMAD3 gene mutation carriers coming to our centre and their families were investigated prospectively with a structured panel including standardized clinical workup, blood tests, total body computed tomography, joint X-rays. Electroneuromyography was performed in selected cases.

Results: Thirty-four SMAD3 gene mutation carriers coming to our centre were identified and 16 relatives were considered affected because of aortic surgery or sudden death (total 50 subjects). Aortic disease was present in 72%, complicated with aortic dissection, surgery or sudden death in 56% at a mean age of 45 years. Aneurysm or tortuosity of the neck arteries was present in 78%, other arteries were affected in 44%, including dissection of coronary artery. Overall, 95% of mutation carriers displayed either aortic or extra-aortic arterial disease. Acrocyanosis was also present in the majority of patients. Osteoarticular manifestations were recorded in all patients. Joint involvement could be severe requiring surgery in young patients, of unusual localization such as tarsus or shoulder, or mimicking crystalline arthropathy with fibrocartilage calcifications. Sixty eight percent of patients displayed neurological symptoms, and 9 suffered peripheral neuropathy. Electroneuromyography revealed an axonal motor and sensory neuropathy in 3 different families, very evocative of type II Charcot-Marie-Tooth (CMT2) disease, although none had mutations in the known CMT2 genes. Autoimmune features including Sjogren’s disease, rheumatoid arthritis, Hashimoto’s disease, or isolated autoantibodies were found in 36% of patients.

Interpretation: SMAD3 gene mutations are associated with aortic dilatation and osteoarthritis, but also autoimmunity and peripheral neuropathy which mimics type II Charcot-Marie-Tooth.

Introduction

Thoracic aortic aneurysms and dissections (TAAD) can occur as inherited mendelian diseases, appearing either as isolated events or associated within a spectrum of clinical features that define various syndromes. The oldest known syndrome is Marfan syndrome that associates TAAD with ocular alterations and other systemic features, listed in the recently modified Ghent nosology [1]. Marfan syndrome is mostly caused by mutations within the gene encoding fibrillin 1 (FBN1) [2,3], the major component of
connective tissue microfibrils. More recently TAAD were also linked to altered TGF-β signaling through the canonical Smad pathway. Indeed, the Loey Dietz syndrome is linked with mutations within the genes encoding TGF-β receptors type 1 or II (TGFBR1 or TGFBR2) [4]. Subsequently, mutations in the SMAD3 [5] and TGFBR2 [6,7] genes were identified. Mutations in the gene encoding Smad3 in autosomal dominant TAAD patients were recently associated with early onset osteoarthritis, defining a new entity: Aneurysms Osteoarthritis Syndrome (AOS) [5]. Such results were confirmed in subsequent reports [8,9,10,11].

The objective of our study was to assess the full spectrum of clinical involvement in newly identified patients carrying a SMAD3 gene mutation. Herein we report that mutations in this gene lead to an extended and more complex syndrome than previously recognized. The phenotypic features encompasses neurological alterations similar to those observed in the axonal type 2 form of Charcot-Marie-Tooth disease (CMT2) and include autoimmune manifestations.

Materials and Methods

Clinical Evaluation

All patients originated from the French National Reference Centre for Marfan Syndrome and related disorders. They were selected after gene screening showing that they carried a disease-causing mutation in the SMAD3 gene. Family members were approached through the index case and invited to attend the National Reference Centre.

All patients initially had been evaluated by geneticists, rheumatologists or paediatricians (depending on their age), cardiologists and ophthalmologists to rule out syndromic forms of TAAD. Slit-lamp examination and cardiac ultrasonography were performed in all subjects. Once results of gene screening were available, all SMAD3 gene mutation carriers were asked to undergo an extended workup provided they gave their written informed consent for participation in this clinical and genetic study in agreement with the requirements of French regulations (Accepted by “Comité de Protection des Personnes CPP Ile de France XI”, 78105 St Germain en Laye). For the purpose of the study and to ensure homogeneity, all retrospective clinical data were reassessed by one physician.

Comprehensive clinical examination used a structured evaluation form exploring each organ.

Laboratory tests included routine blood biology (hemogram, C reactive protein, prothrombin time, partial-thromboplastin time, sodium, potassium, chloride, calcium, phosphate, ura nitrogen, creatinin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, lipase, lactate dehydrogenase, creatinin kinase, serum electrophoresis), autoantibodies (antinuclear antibodies, anticytoplasmic antibodies, antinuclear antibodies, anti-SS-A and anti-SS-B, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, antinuclear antibodies, anti-DNA, 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ENMG was proposed to all patients with neurological examination abnormality, and performed in 9 who agreed.

Cardio-Vascular Features

**Aorta and mitral.** Aortic features were studied in all 50 subjects (26 mutation carriers and 24 obligate mutation carriers) (Figure 2). Disease of the thoracic ascending aorta was present in 36/50 (dilatation leading or not to surgery, aortic dissection or sudden death), and led to an aortic event in 28 (aortic dissection, preventive aortic surgery and/or sudden death) at a mean age of 44.8 years. Thoracic aortic dilatation was evidenced in 30% of the non-operated patients (8/26), maximal at the level of the sinuses of Valsalva. Four patients (8%, 4/50) had a history of abdominal aortic dissection, isolated in one patient. Overall, 54% (28/50) displayed documented aortic disease (aneurysm, dissection, surgery) and an additional 18% (9/50) sudden death of unknown origin (total 74% [37/50]).

Finally, mitral valve prolapse with mild mitral regurgitation was noted in 6 unoperated patients.

**Other arteries and cardiac findings.** Overall, 78% of the patients (14/18) had a dissection, aneurysm or tortuosity of neck arteries evidenced by a CT-scanner (Table 2). Furthermore, in

| Family  | Exon    | Nucleotide       | Variation                  |
|---------|---------|------------------|----------------------------|
| BIC5301 | Exon 6  | c.733G>A         | p.Gly245Arg                |
| BIC3521 | Exon 6  | c.742T>C         | p.Phe248Leu                |
| BIC4191 | Exon 6  | c.860G>A         | p.Arg287Gln                |
| BIC886  | Exon 6  | c.688delC        | p.Pro223Glnfs*18           |
| BIC792  | Exon 6  | c.862_871dup11[AGACACATCGG] | p.Arg292Aspf*53       |
| BIC873  | Exon 8  | c.1102G>T        | p.Arg368*                  |
| BIC029  | Exon 9  | c.1179_1180dupC  | p.Cys394Leufs*4            |
| BIC915  | Exon 9  | c.1267A>G        | p.Ser423Gly                |

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44% (8/18), involvement of other medium-sized arteries (subclavicular, renal, splenic or iliac) was diagnosed. All were asymptomatic, except for dissection of a coronary artery in one 58 years-old woman who suffered acute myocardial infarction. Interestingly, in this patient, CT-scanner also revealed multiple asymptomatic dissections of subclavian, carotid, vertebral, renal, and mesenteric arteries and no aortic dilatation. No cerebral artery aneurysm was found on CT scanner in our population.

Overall, 95% (18/19) of the SMAD3 mutation carriers who had been explored by CT-scanner displayed either aortic or extra-aortic vascular disease.

Furthermore, microvasculature was also involved. Raynaud phenomenon, with typical tricolor response, was found in 3/22 subjects (14%), while acrocyanosis (without pallor) was present in 11/22 patients (52%). No patient had digital ulcers. Since Raynaud phenomenon or acrocyanosis are frequent in the general population, we evaluated its prevalence in consecutive subjects attending the National Reference Center from October to December 2011 (Table 3). Prevalence of acrocyanosis was significantly greater in SMAD3 gene mutation carriers as compared to healthy subjects (p = 0.005, OR = 6.7 [1.6–25.5]) or Marfan patients carrying a FBN1 mutation (p = 0.006, OR = 4.3 [1.4–15.5]) (Table 3).

**Neurological Features**

Overall, 68% (15/22) of patients belonging to 6 different families, displayed neurological symptoms such as muscle cramps, paresthesia, hypoesthesia, or gait disturbance (Table 3). Among these symptomatic patients, 9/15 belonging to 5 families had clinical evidence for peripheral neuropathy including absent tendon reflexes (n = 5), proprioceptive sensory loss (n = 1), recurrent sprain (n = 9) or pes cavus (n = 6). Three patients - belonging to 3 independent families – complained from severe paresthesia starting at adolescence. In the 3 patients, examination showed distal sensory loss and abolished tendon reflexes in lower limbs. All 3 patients had pes cavus and scoliosis. Electroneuromyography disclosed a pattern of axonal motor and sensory neuropathy in the 3 patients, including radicular denervation in 2 patients, in left S1 radicular nerve and in left L5 radicular nerve, respectively. Nerve conduction velocities were reduced in peroneal nerve with reduction of the compound muscle action potential amplitude and prolonged distal latency in two. Peroneal sensory nerve action potential was also decreased in all patients while sural sensory nerve action potential was decreased in two. Finally, compound muscle action potential amplitudes, distal latency and
conduction velocity were normal in upper limbs in all patients (Table 5). In view of these results, ENMG was performed in 6 additional patients; only one displayed similar results. Hence, a total of 4/9 patients displayed a pattern of chronic sensory and motor neuropathy with decreased conduction velocity and compound muscle action potential amplitudes, consistent with CMT2-like neuropathy.

The association of an axonal neuropathy with high arches and scoliosis was suggestive of axonal type 2 form of Charcot-Marie-Tooth disease (CMT2). CMT2 is a highly heterogeneous genetic disorder but 4 genes are the most frequently involved: the GJB1 (connexin-32), MPZ (P0 or myelin protein-zero), MFN2 (mitofusin 2) and GDAP1 (ganglioside-induced differentiation-associated protein 1). To exclude a second gene defect, these genes were screened and no mutation was found. Conversely, SMAD3 gene was screened in 70 patients with an autosomal dominant CMT2 but with no mutation in the major known CMT2-related genes (French National Reference Center, La Pitié Salpêtrière hospital, neurology department). No mutation was found in the SMAD3 gene in these patients.

### Table 2. Cardiovascular features.

| Disease of the ascending aorta | Percentage | Number | Mean age | Age range |
|--------------------------------|------------|--------|----------|-----------|
| Uncomplicated dilatation >2 DS | 72%        | 36/50  | -        | -         |
| Preventive surgery             | 30%        | 8/26   | -        | 3–83      |
| Ascending aorta dissection     | 14%        | 7/50   | 46.0     | 27–58     |
| Sudden death                   | 26%        | 13/50  | 46.6     | 30–68     |
| Initial abdominal aorta dissection | 30%        | 15/50  | 43.3     | 22–68     |

| Disease of the neck arteries   | Percentage | Number | Mean age | Age range |
|--------------------------------|------------|--------|----------|-----------|
| Dissection                     | 78%        | 14/18  | -        | -         |

| Carotid arteries               | Percentage | Number | Mean age | Age range |
|--------------------------------|------------|--------|----------|-----------|
| Dissection                     | 6%         | 1/18   | -        | -         |
| Aneurysm                       | 33%        | 6/18   | -        | -         |
| Tortuosity                     | 72%        | 13/18  | -        | -         |

| Vertebral arteries             | Percentage | Number | Mean age | Age range |
|--------------------------------|------------|--------|----------|-----------|
| Dissection                     | 6%         | 1/18   | -        | -         |
| Aneurysm                       | 22%        | 4/18   | -        | -         |
| Tortuosity                     | 22%        | 4/18   | -        | -         |

| Disease of other arteries      | Percentage | Number | Mean age | Age range |
|--------------------------------|------------|--------|----------|-----------|
| Dissection                     | 44%        | 8/18   | -        | -         |
| Aneurysm                       | 39%        | 7/18   | -        | -         |
| Tortuosity                     | 11%        | 2/18   | -        | -         |

### Table 3. Acrocyanosis, cramps and joint pains prevalence in SMAD3 mutation-carriers and control patients (Cont).

| ACROCYANOSIS | CRAMPS | JOINT PAINS |
|--------------|--------|-------------|
| SMAD3 vs FBN1 (2/129) | p = 4.30.10−4 | p = 0.46 | 0.0055 |
| Odd Ratio | 9.12 [2.77; 32.91] | 10.77 [3.62; 35.36] |
| SMAD3 vs Cont (22/33) | p = 0.0046 | p = 0.067 | 0.0023 |
| Odd Ratio | 6.71 [1.57; 35.41] | 6.18 [1.68; 25.54] |
| SMAD3 vs ALL (22/198) | p = 1.02.10−4 | p = 0.56 | 0.0217 |
| Odd Ratio | 6.83 [2.41; 19.48] | 2.47 [1.06; 5.68] |

Autoimmunity

Eight patients (8/22 = 36%) presented with autoimmune features. One patient had primary Sjogren’s disease, defined by sicca syndrome, lymphocytic sialadenitis, anti Ro (SSA) antibodies, and numerous asymptomatic pulmonary cysts. One patient suffered clear-cut rheumatoid arthritis, with chronic arthritis in both hands and positive rheumatoid factor. Two patients - a 66 years-old woman treated for hypothyroidism and a 50 years-old man with prominent goitre with raised anti thyroperoxidase antibodies serum levels in both cases – suffered Hashimoto disease. Four patients had isolated – i.e. without clinical manifestations – autoantibodies: anti-nuclear antibody and anti- SSB/La, anti-citrullinated protein, anti-cardiolipin and anti-nuclear antibodies only, respectively.

Allergy

Eleven patients (11/22 = 50%) displayed allergic manifestations such as asthma (n = 4), allergic rhinitis (n = 5), allergic conjuncti-
### Table 4. Rheumatologic manifestations.

| Clinical manifestations | 70% | 16/23 | 22% | 5/23 | 17% | 4/23 | 39% | 9/23 | 17% | 4/23 | 74% | 17/23 |
|-------------------------|-----|-------|-----|------|-----|------|-----|------|-----|------|-----|-------|
| Peripheral pain         |     |       |     |      |     |      |     |      |     |      |     |       |
| Peripheral deformity    |     |       |     |      |     |      |     |      |     |      |     |       |
| Hyperlaxity             |     |       |     |      |     |      |     |      |     |      |     |       |
| Sprain                  |     |       |     |      |     |      |     |      |     |      |     |       |
| Spinal : cervical pain  |     |       |     |      |     |      |     |      |     |      |     |       |
| Spinal : lumbar pain    |     |       |     |      |     |      |     |      |     |      |     |       |

### Radiological manifestations

|                | 93% | 13/14 | 85% | 17/20 | 50% | 9/18 | 4%  | 1/23 | 22% | 2/9  |
|----------------|-----|-------|-----|-------|-----|------|-----|------|-----|------|
| Peripheral osteoarthritis |   |   |   |   |   |   |   |   |   |   |
| Spinal osteoarthritis    |   |   |   |   |   |   |   |   |   |   |
| Scoliosis                |   |   |   |   |   |   |   |   |   |   |
| Osteochondritis          |   |   |   |   |   |   |   |   |   |   |
| Scheuerman               |   |   |   |   |   |   |   |   |   |   |

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### Table 5. Electroneuromyography in patients with severe neurologic symptoms.

|                         | Patient 1 | Patient 2 | Patient 3 |
|-------------------------|-----------|-----------|-----------|
| Pes cavus               | Yes       | Yes       | No        |
| Scoliosis               | Yes       | Yes       | No        |
| Achille tendon reflex   | Not obtained | Not obtained | Normal   |
| Rotulian tendon reflex  | Not obtained | Not obtained | Normal   |
| Distal sensory loss     | Yes       | Yes       | Yes       |
| Vibration at the wrist  | Decreased | Normal    | Decreased |
| CMAP amplitude (peroneal) | Decreased | Decreased (R) | Decreased (L) |
| R                       | 1.39 mV   | 2.07 mV   | 5.91 mV   |
| L                       | 1.41 mV   | 6.04 mV   | 4.1 mV    |
| DL (peroneal)           | Limit     | Prolonged (R) | Prolonged (L) |
| R                       | 4.9 ms    | 5.6 ms    | 4.5 ms    |
| L                       | 4.9 ms    | 3.6 ms    | 5.6 ms    |
| CV (peroneal)           | Decreased | Decreased | Decreased |
| R                       | 37 m/s    | 35 m/s    | 41 m/s    |
| L                       | 37 m/s    | 30 m/s    | 37 m/s    |
| CMAP amplitude (tibial post) | Decreased | Normal    | Normal    |
| R                       | 2.20 mV   | 6.77 mV   | 12.66 mV  |
| L                       | 0.81 mV   | 8.85 mV   | 13.65 mV  |
| Sural SNAP              | Decreased (L) | Decreased | Normal    |
| R                       | 13.6 µV   | 4.5 µV    | 17.4 µV   |
| L                       | 5.7 µV    | 5.7 µV    | 17.0 µV   |
| Peroneal SNAP           | Not obtained | Decreased | Decreased |
| R                       | 2.3 µV    | 6.9 µV    |
| L                       | 3.3 µV    | 6.2 µV    |
| Upper limbs CV          | Normal    | Normal    | Normal    |
| Upper limbs CMAP        | Normal    | Normal    | Normal    |
| Upper limbs SNAP        | Normal    | Normal    | Normal    |
| Lower limbs detection   | Chronic neurogenic pattern | Normal | Normal |
| Upper limbs detection   | Normal    | Normal    | Normal    |

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vitis (n = 5), angiodema (n = 1), food allergy (n = 1) or eczema (n = 4).

Marfan Criteria
No ectopia lenti was found among SMAD3 gene mutation carriers. Skeletal features were heterogeneous and Ghent 2 systemic scores ranged from 2 to 11.

Discussion
We report on new findings in patients with SMAD3 gene mutations that extend the clinical spectrum beyond aortic dilatation and osteoarthritis which were previously reported [5,8,9,10,11]. Neurological symptoms such as muscle cramps, paresthesia, hypoesthesia and gait disturbance were observed in the majority of SMAD3 gene mutation carriers, half presenting an objective neuropathy. Three families out of 8 displayed a CMT2-like neuropathy on electromyography but none had mutations in the major genes usually responsible for CMT2. Because of the very low frequency (1/2500) of CMT2 in the general population [14], such results strongly suggest that SMAD3 gene mutations are responsible for the CMT2-like phenotype observed in our patients. Of note, SMAD3 gene mutations were not found in 70 CMT2 proband samples which were selected through a tertiary neurological centre. Interestingly, aorta imaging has not yet been performed in patients with CMT2 and no known specific mutations. Although TGF beta signalling may interfere with axon development [15] the pathogenetic mechanism of neurological involvement in our patients is unknown.

We also observed auto-immune features in 36% (8/22) of patients, including Sjögren’s syndrome, rheumatoid arthritis, and Hashimoto disease. The role of the TGF beta pathway in the maintenance of peripheral tolerance in T-cells is well established [16]. Furthermore, transgenic mouse model with Tgfr2 conditional knock-out in dendritic cells develop multiorgan autoimmunity and premature thymus involution [17]. Hence, SMAD3 gene mutations are expected to affect self-tolerance. Patients carrying mutations in other genes altering TGF beta signaling (TGFB1, TGFB2, TGFBR2) may also warrant screening for autoimmune dysregulation [18].

Fifty percent of SMAD3 mutation carriers suffered of allergic disease, especially asthma (23%) and allergic conjunctivitis (23%). Of note, a recent study suggested that mutations in genes encoding TGF beta receptor subunits may predispose to allergic disease [19].

In SMAD3 mutation carriers, arterial disease is centered by TAA as an established predominant and life-threatening manifestation, observed in 72% of our patients (36/50). Neck arteries were also affected in 70% of the subjects (arterial tortuosity, dissection or aneurysm) and, unexpectedly, coronary and/or digestive arteries in 44% of the patients. In our cohort, CT-scanners of aorta and large arteries had a sensitivity of 95% (18/19) for diagnosis of the disease. Finally, Raynaud syndrome or acrocyanosis were observed in half of the SMAD3 mutation carriers (significantly more frequently than in normal subjects or Marfan controls), suggesting also microvasculature involvement.

As expected from previous reports on SMAD3 mutation carriers [5,8,9,10,11], we observed skeletal involvement in 100% of the subjects on X-ray or CT-scan study. Joint pain was significantly more frequent in SMAD3 mutation carriers, as compared to both normal subjects or Marfan patients, even in the youngest patients. Joint involvement could be severe and treated with surgery in young patients, of unusual localization such as tarsus or shoulder, or mimicking crystalline arthropathy with calcifications and narrowed joint spaces. Because of the possibility of an underlying widespread vascular disease, we believe that aorta and arteries study should be discussed in patients suffering atypical osteoarthritis, either unusually severe, of early onset, or with atypical localization.

In conclusion, type 2 Charcot Marie Tooth-like neuropathy and autoimmune may be added to the previously described atypical osteoarthropathy and life-threatening aortic disease in the clinical spectrum associated with SMAD 3 gene mutation.

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Author Contributions
Conceived and designed the experiments: TP GJ CB. Performed the experiments: MA DG CM DD JR VC BG AMV JSL PR GJ. Analyzed the data: MA DG FAC GJ CB EL. Contributed reagents/materials/analysis tools: MA CD TP CB GJ. Wrote the paper: MA DG FAC TP CB GJ.

References
1. Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, et al. (2010) The revised Ghent nosology for the Marfan syndrome. J Med Genet 47: 476–485.
2. Dietz HC, Cutting GR, Pyeritz RE, Maden CL, Sakai LY, et al. (1991) Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 352: 337–339.
3. Faivre L, Collod-Beroud G, Loeys BL, Child A, Binquet C, et al. (2007) Effect of gene mutations in TGFB2 cause a syndromic presentation of thoracic aortic aneurysm. Nat Genet 44: 922–927.
4. van der Linde D, van de Laar IM, Bertoli-Avella AM, Oldenburg RA, Bekkers JA, et al. (2012) Aneurysm-osteoarthritis syndrome with visceral and iliac artery involvement. J Med Genet 49: 47–37.
5. van der Linde D, Verhaegen HJ, Moelker A, van der Laar IM, Van Herzele L, et al. (2013) Aneurysm-osteoarthritis syndrome with visceral and iliac artery aneurysms. J Vasc Surg 57: 96–102.
6. Roman MJ, Devereux RB, Kramer-Fox R, O’Laughlin J (1989) Two-dimensional echocardiographic aortic root dimensions in normal children and adults. Am J Cardiol 64: 507–512.
7. Levine RA, Stathogiannis E, Newell JB, Harrigan P, Weyman AE (1988) Consideration of echocardiographic standards for mitral valve prolapse: lack of agreement among leaflet displacement isolated to the apical four chamber view and independent echocardiographic evidence of abnormality. J Am Coll Cardiol 11: 1010–1019.
8. Skre H (1974) Genetic and clinical aspects of Charcot-Marie-Tooth’s disease. Clin Genet 6: 58–118.
15. Yi, J.J., Barnes, A.P., Hand, R., Polleux, F., Ehlers, M.D. (2010) TGF-beta signaling specifies axons during brain development. Cell 142: 144–157.

16. Li, M.O., Wan, Y.Y., Sanjabi, S., Robertson, A.K., Flavell, R.A. (2006) Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 24: 99–146.

17. Ramalingam, R., Larmonier, C.B., Thurston, R.D., Midura-Kiela, M.T., Zheng, S.G., et al. (2012) Dendritic cell-specific disruption of TGF-beta receptor II leads to altered regulatory T cell phenotype and spontaneous multiorgan autoimmunity. J Immunol 189: 3078–3093.

18. Felgentreff, K., Siepe, M., Kotthoff, S., von Kodolitsch, Y., Schachtrop, K., et al. (2014) Severe eczema and Hyper-IgE in Loeys-Dietz syndrome - contribution to new findings of immune dysregulation in connective tissue disorders. Clin Immunol 150: 43–50.

19. Frischmeyer-Guerrerio, P.A., Guerrerio, A.L., Oswald, G., Chichester, K., Myers, I., et al. (2013) TGFbeta receptor mutations impose a strong predisposition for human allergic disease. Sci Transl Med 5: 195ra194.