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Mutations in the cyclin family member *FAM58A* cause an X-linked dominant disorder characterized by syndactyly, telecanthus and anogenital and renal malformations

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We identified four girls with a consistent constellation of facial dysmorphism and malformations previously reported in a single mother–daughter pair. Toe syndactyly, telecanthus and anogenital and renal malformations were present in all affected individuals; thus, we propose the name 'STAR syndrome' for this disorder. Using array CGH, qPCR and sequence analysis, we found causative mutations in *FAM58A* on Xq28 in all affected individuals, suggesting an X-linked dominant inheritance pattern for this recognizable syndrome.

We identified four unrelated girls with anogenital and renal malformations, dysmorphic facial features, normal intellect and syndactyly of toes. A similar combination of features had been reported previously in a mother–daughter pair[1] (**Table 1** and **Supplementary Note** online). These authors noted clinical overlap with Townes-Brocks syndrome but suggested that the phenotype represented a separate autosomal dominant entity (MIM601446). Here we define the cardinal features of this syndrome as a characteristic facial appearance with apparent telecanthus and broad tripartite nasal tip, variable syndactyly of toes 2–5, hypoplastic labia, anal atresia and urogenital malformations (**Fig. 1a–h**). We also observed a variety of other features (**Table 1**).

On the basis of the phenotypic overlap with Townes-Brocks, Okihiro and Feingold syndromes, we analyzed *SALL1* (ref. 2), *SALL4* (ref. 3) and *MYCN*[4] but found no mutations in any of these genes (**Supplementary Methods** online). Next, we carried out genome-wide high-resolution oligonucleotide array comparative genomic hybridization (CGH)[5] analysis (**Supplementary Methods**) of genomic DNA from the most severely affected individual (case 1, with lower lid coloboma, epilepsy and syringomyelia) and identified a heterozygous deletion of 37.9–50.7 kb on Xq28, which removed exons 1 and 2 of *FAM58A* (**Fig. 1i,j**). Using real-time PCR, we confirmed the deletion in the child and excluded it in her unaffected parents (**Supplementary Fig. 1a** online, **Supplementary Methods** and **Supplementary Table 1** online). Through CGH with a customized oligonucleotide array enriched in probes for Xq28, followed by breakpoint cloning, we defined the exact deletion size as 40,068 bp (g.152,514,164_152,554,231del(chromosome X, NCBI Build 36.2); **Fig. 1j** and **Supplementary Figs. 2,3** online). The deletion removes the coding regions of exons 1 and 2 as well as intron 1 (2,774 bp), 492 bp of intron 2, and 36,608 bp of 5′ sequence, including the 5′ UTR and the entire *KRT18P48* pseudogene (NCBI gene ID 340598). Paternity was proven using routine methods. We did not find deletions overlapping *FAM58A* in the available copy number variation (CNV) databases.

Subsequently, we carried out qPCR analysis of the three other affected individuals (cases 2, 3 and 4) and the mother-daughter pair from the literature (cases 5 and 6). In case 3, we detected a *de novo* heterozygous deletion of 1.1–10.3 kb overlapping exon 5 (**Supplementary Fig. 1b** online). Using Xq28-targeted array CGH and breakpoint cloning, we identified a deletion of 4,249 bp (g.152,504,123_152,508,371del(chromosome X, NCBI Build 36.2); **Fig. 1j** and **Supplementary Figs. 2,3**), which removed 1,265 bp of intron 4, all of exon 5, including the 3′ UTR, and 2,454 bp of 3′ sequence.

We found heterozygous *FAM58A* point mutations in the remaining cases (**Fig. 1j**, **Supplementary Fig. 2**, **Supplementary Methods** and **Supplementary Table 1**). In case 2, we identified the mutation 555+1G>A, affecting the splice donor site of intron 4. In case 4, we identified the frameshift mutation 201dupT, which immediately results in a premature stop codon N68XfsX1. In cases 5 and 6, we detected the mutation 556-1G>A, which alters the splice acceptor site of intron 4. We validated the point mutations and deletions by independent rounds of PCR and sequencing or by qPCR. We confirmed paternity and *de novo* status of the point mutations and deletions in all sporadic cases. None of the mutations were seen in the DNA of 60 unaffected female

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Table 1 Clinical features in STAR syndrome cases

Feature	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Feingold syndrome	Okihiro syndrome	Townes-Brocks syndrome
Telecanthus	X	X	X	X	No	No			
Lop ears	X	X	X	X	No	No (low set)		(X)	X
Clinodactyly 5th finger	X	X	X	X	X	X	X		
Syndactyly of toes (not 2–3)	X	X	X	X	X	X	X	Rare	Occasional
Anal atresia	X	X	X	X	X	X	Reported in 1 individual	Occasional	X
Genital anomaly (external)	Hpl. labia	Hpl. labia	Hpl. labia	Hpl. labia	Clitoromeg.	Clitoromeg.			Rare
Genital anomaly (internal)	Dupl. vagina + uterus		Dupl. vagina, bic. uterus		None	None			Rare
Renal anomaly			Sol. kidney	Cros. fused kidneys	Pelv. kidney, ESRD	Sol. kidney, ESRD	Rare	Occasional	X
Urinary tract anomaly	VU reflux	Hydronephr., VU reflux, megaureter		VU reflux	Hydronephr., small bladder	VU reflux			
Radial ray anomaly			X					X	X
Congenital heart disease		PFO, peripheral pulm. artery stenosis	Bicusp. aortic valve, valv. pulm. stenosis	ASD			Rare	Occasional	Occasional
Height (percentile)	3rd	<3rd	<3rd	5th	<3rd	<3rd			
Eyes		Macular hypoplasia	Dystr. retina, –5D myopia	Normal					
Craniosynostosis	Cor., lambd.		sagittal						

Abbreviations: asym., asymmetrical; bic., bicornuate; bicusp., bicuspid; bif., bifid; clitoromeg., clitoromegaly; cor., coronal; cros., crossed; dupl., duplicated; ESDR, end-stage renal disease; hpl., hypoplastic; hydronephr., hydronephrosis; lambd., lambdoid; PFO, persistent formane ovale; pelv., pelvic; pulm., pulmonary; sol., solitary; valv., valvular; VU, vesicourethral. An "X" denotes a trait present in the respective case or typically observed in the overlapping syndromes. More detailed information, especially on cases 5 and 6, is contained in the **Supplementary Note**.

controls, and no larger deletions involving *FAM58A* were found in 93 unrelated array-CGH investigations.

By analyzing X-chromosome inactivation (**Supplementary Methods** and **Supplementary Fig. 4** online), we found complete skewing of X inactivation in cases 1 and 3–6 and almost complete skewing in case 2, suggesting that cells carrying the mutation on the active X chromosome have a growth disadvantage during fetal development. Using RT-PCR on RNA from lymphoblastoid cells of case 2 (**Supplementary Fig. 2**), we did not find any aberrant splice products as additional evidence that the mutated allele is inactivated. Furthermore, *FAM58A* is subjected to X inactivation[6]. In cases 1 and 3, the parental origin of the deletions could not be determined, as a result of lack of informative SNPs. Case 5, the mother of case 6, gave birth to two boys, both clinically unaffected (samples not available). We cannot exclude that the condition is lethal in males. No fetal losses were reported from any of the families.

The function of *FAM58A* is unknown. The gene consists of five coding exons, and the 642-bp coding region encodes a protein of 214 amino acids. GenBank lists a mRNA length of 1,257 bp for the reference sequence (NM_152274.2). Expression of the gene (by EST data) was found in 27 of 48 adult tissues including kidney, colon, cervix and uterus, but not heart (NCBI expression viewer, UniGene Hs.496943). Expression was also noted in 24 of 26 listed tumor tissues as well as in embryo and fetus. Genes homologous to *FAM58A* (NCBI HomoloGene: 13362) are found on the X chromosome in the chimpanzee and the dog. The zebrafish has a similar gene on chromosome 23. However, in the mouse and rat, there are no true homologs. These species have similar but intronless genes on chromosomes 11 (mouse) and 10 (rat), most likely arising from a retrotransposon insertion event. On the murine X chromosome, the flanking genes *Atp2b3* and *Dusp9* are conserved, but only remnants of the *FAM58A* sequence can be detected.

FAM58A contains a cyclin-box-fold domain, a protein-binding domain found in cyclins with a role in cell cycle and transcription control. No human phenotype resulting from a cyclin gene mutation has yet been reported. Homozygous knockout mice for *Ccnd1* (encoding cyclin D1) are viable but small and have reduced lifespan. They also have dystrophic changes of the retina, likely as a result of decreased cell proliferation and degeneration of photoreceptor cells during embryogenesis[7,8].

Cyclin D1 colocalizes with SALL4 in the nucleus, and both proteins cooperatively mediate transcriptional repression[9]. As the phenotype of our cases overlaps considerably with that of Townes-Brocks syndrome caused by *SALL1* mutations[1], we carried out co-immunoprecipitation to find out if SALL1 or SALL4 would interact with FAM58A in a manner similar to that observed for SALL4 and cyclin D1. We found that FAM58A interacts with SALL1 but not with SALL4 (**Supplementary Fig. 5** online), supporting the hypothesis that *FAM58A* and *SALL1* participate in the same developmental pathway.

How do *FAM58A* mutations lead to STAR syndrome? Growth retardation (all cases; **Table 1**) and retinal abnormalities (three cases) are reminiscent of the reduced body size and retinal anomalies in cyclin D1 knockout mice[7,8]. Therefore, a proliferation defect might be partly responsible for STAR syndrome. To address this question, we carried out a knockdown of *FAM58A* mRNA followed by a proliferation assay. Transfection of HEK293 cells with three different *FAM58A*-specific RNAi oligonucleotides resulted in a significant reduction of both *FAM58A* mRNA expression and proliferation of transfected cells (**Supplementary Methods** and **Supplementary Fig. 6** online), supporting the link between FAM58A and cell proliferation.

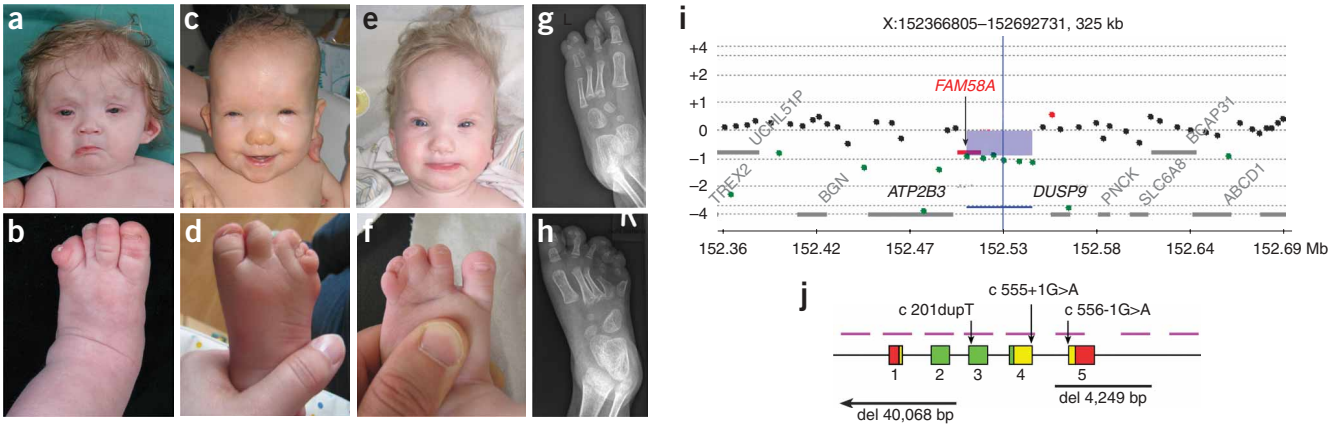

Figure 1 Clinical and molecular characterization of STAR syndrome. (**a**–**f**) Facial appearances of cases 1–3 (apparent telecanthus, dysplastic ears and thin upper lips; **a**,**c**,**e**), and toe syndactyly 2–5, 3–5 or 4–5 (**b**,**d**,**f**) in these cases illustrate recognizable features of STAR syndrome (specific parental consent has been obtained for publication of these photographs). Anal atresia and hypoplastic labia are not shown. (**g**,**h**) X-ray films of the feet of case 2 showing only four rays on the left and delta-shaped 4th and 5th metatarsals on the right (**h**; compare to clinical picture in **d**). (**i**) Array-CGH data. Log_2 ratio represents copy number loss of six probes spanning between 37.9 and 50.7 kb, with one probe positioned within *FAM58A*. The deletion does not remove parts of other functional genes. (**j**) Schematic structure of *FAM58A* and position of the mutations. *FAM58A* has five coding exons (boxes). The cyclin domain (green) is encoded by exons 2–4. The horizontal arrow indicates the deletion extending 5′ in case 1, which includes exons 1 and 2, whereas the horizontal line below exon 5 indicates the deletion found in case 3, which removes exon 5 and some 3′ sequence. The pink horizontal bars above the boxes indicate the amplicons used for qPCR and sequencing (one alternative exon 5 amplicon is not indicated because of space constraints). The mutation 201dupT (case 4) results in an immediate stop codon, and the 555+1G>A and 555-1G>A splice mutations in cases 2, 5 and 6 are predicted to be deleterious because they alter the conserved splice donor and acceptor site of intron 4, respectively.

We found that loss-of-function mutations of *FAM58A* result in a rather homogeneous clinical phenotype. The additional anomalies in case 1 are likely to result from an effect of the 40-kb deletion on expression of a neighboring gene, possibly *ATP2B3* or *DUSP9*. However, we cannot exclude that the homogeneous phenotype results from an ascertainment bias and that *FAM58A* mutations, including missense changes, could result in a broader spectrum of malformations. The genes causing the overlapping phenotypes of STAR syndrome and Townes-Brocks syndrome seem to act in the same pathway. Of note, *MYCN*, a gene mutated in Feingold syndrome, is a direct regulator of cyclin D2 (refs. 10,11); thus, it is worth exploring whether the phenotypic similarities between Feingold and STAR syndrome might be explained by direct regulation of *FAM58A* by *MYCN*.

FAM58A is located approximately 0.56 Mb centromeric to *MECP2* on Xq28. Duplications overlapping both *MECP2* and *FAM58A* have been described and are not associated with a clinical phenotype in females[12], but no deletions overlapping both *MECP2* and *FAM58A* have been observed to date[13]. Although other genes between *FAM58A* and *MECP2* have been implicated in brain development, *FAM58A* and *MECP2* are the only genes in this region known to result in X-linked dominant phenotypes; thus, deletion of both genes on the same allele might be lethal in both males and females.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

S.U. contributed to the clinical evalutation of cases, syndrome delineation and subject enrollment in the study. D.B. performed array CGH, mutation analysis and qPCR. W.B. performed mutation analysis on *MYCN*, *SALL1* and *SALL4* and *FAM58A* breakpoint cloning. F.J.K. performed co-immunoprecipitation studies. K. Buiting performed X-chromosome inactivation studies. S.K. and P.B. performed cell culture studies, siRNA knockdown experiments and proliferation assays, and contributed to the manuscript. J.B., F.B. and A.C. cloned expression constructs and performed RT-PCR. K. Borowski, K.K.-N., G.M., T.S.-M., B.S., D. Bartholdi, R.S., B.Z., and A.S.-F contributed to subject enrollment and clinical evaluation. S.U., D.B., J.B., F.J.K., K.Bu., S.K., P.B., R.S., G.M. and A.S.-F. also contributed to the manuscript. J.K. oversaw all aspects of the research and wrote major parts of the manuscript.

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