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MUTATION UPDATE

SALL4 Mutations in Okihiro Syndrome (Duane-Radial Ray Syndrome), Acro-Renal-Ocular Syndrome, and Related Disorders

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Okihiro/Duane-radial ray syndrome (DRRS) is an autosomal dominant condition characterized by radial ray defects and Duane anomaly (a form of strabismus). Other abnormalities reported in this condition are anal, renal, cardiac, ear, and foot malformations, and hearing loss. The disease is the result of a mutation in the *SALL4* gene, a human gene related to the developmental regulator *spalt* (*sal*) of *Drosophila melanogaster*. *SALL4* mutations may also cause acro-renal-ocular syndrome (AROS), which differs from DRRS by the presence of structural eye anomalies, and phenotypes similar to thalidomide embryopathy and Holt-Oram syndrome (HOS). The *SALL4* gene product is a zinc finger protein that is thought to act as a transcription factor. It contains three highly conserved C2H2 double zinc finger domains, which are evenly distributed. A single C2H2 motif is attached to the second domain, and at the amino terminus SALL4 contains a C2HC motif. Seventeen of the 22 *SALL4* mutations known to date (five of which are presented here for the first time) are located in exon 2, and five are located in exon 3. These are nonsense mutations, short duplications, and short deletions. All of the mutations lead to preterminal stop codons and are thought to cause the phenotype via haploinsufficiency. This assumption is supported by the detection of six larger deletions involving the whole gene or single exons. This article summarizes the current knowledge about *SALL4* defects and associated syndromes, and describes the clinical distinctions with similar phenotypes caused by other gene defects. Hum Mutat 26(3), 176–183, 2005. **© 2005 Wiley-Liss, Inc.**

KEY WORDS: SALL4; Okihiro syndrome; acro-renal-ocular syndrome; mutation; zinc finger; transcription factor; haploinsufficiency

INTRODUCTION

The *SALL* genes, derived from the *Drosophila* gene *spalt* (*sal*) [Kühnlein et al., 1994], represent likely zinc-finger transcription factors. Four such genes have been identified, and two of these (*SALL2* [Kohlhase et al., 1996] and *SALL3* [Kohlhase et al., 1999a]) have not yet been associated with human diseases. Mutations in the *SALL1* gene on chromosome 16q12.1 cause Townes-Brocks syndrome (TBS) and related phenotypes [Albrecht et al., 2004; Engels et al., 2000; Kohlhase, 2000; Kohlhase et al., 1998], whereas mutations in the *SALL4* gene have been shown to cause Okihiro/Duane-radial ray syndrome (DRRS; MIM# 607323) [Al-Baradie et al., 2002; Kohlhase et al., 2002b], acro-renal-ocular syndrome (AROS; MIM# 102490), Holt-Oram syndrome (HOS; MIM# 142900), or suspected thalidomide embryopathy [Kohlhase and Holmes, 2004; Kohlhase et al., 2003].

Okihiro syndrome/DRRS (MIM# 607323) is an autosomal dominant condition characterized by radial ray malformations associated with Duane anomaly, a congenital eye movement disorder characterized by limitation of abduction and narrowing of the palpebral fissure with retraction of the globe on adduction [Chun et al., 2001]. Okihiro syndrome has also been described by other authors and is known by other names in the literature. Temtamy et al. [1975] used the term DR syndrome (D representing Duane anomaly and deafness, and R representing the radial and renal manifestations of the condition). The first report may be attributed to Crisp [1918], and another early case was reported by Ferrell et al. [1966].

Familial occurrences of radial ray malformations, which are noteworthy for their variability, in association with Duane eye

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anomaly have been the key features of several reports [Cross and Pfaffenbach, 1972; Cross et al., 2000; Newbury-Ecob et al., 1996; Yang et al., 1990]. The range of abnormalities reported in these patients is extensive and includes anal stenosis, pigmentary disturbances, hearing deficit, renal malformations, external ear malformations, facial asymmetry, and cardiac lesions, particularly atrial septal defect.

Two groups identified mutations in the *SALL4* gene as the cause of Okihiro syndrome. Kohlhase et al. [1999b] noticed the overlap in the clinical manifestations between TBS and Okihiro syndrome, and assumed that mutations in another *SALL* gene could be the cause of Okihiro syndrome [Kohlhase et al., 2002b]. The cloning of the *SALL4* gene, its mapping to chromosome 20q13.13-13.2 [Deloukas et al., 2001], and the observation of similar limb malformations in patients with chromosome 20 deletions encompassing the *SALL4* region [Fraisse et al., 1981; Shabtai et al., 1993] led them to consider *SALL4* as a strong candidate. This was further substantiated by the report of a phenotype with cardiac and limb anomalies, diagnosed as HOS, that is associated with a de novo pericentric inversion of chromosome 20 involving a breakpoint at q13.2 [Yang et al., 1990]. Following this approach, Kohlhase et al. [2002b] were able to demonstrate mutations in five out of eight families with Okihiro syndrome [Kohlhase et al., 2002b]. Al-Baradie et al. [2002] performed genome-wide linkage studies in families with Okihiro syndrome, resulting in a 21.6 cM linkage interval, and decided to screen the *SALL4* gene for mutations. As a result, mutations were detected in three different families.

In reviewing the mutations in the *SALL4* gene associated with Okihiro syndrome, Kohlhase et al. [2002b] detected a variety of abnormalities, including choanal atresia, triphalangeal thumb, renal agenesis, unilateral deafness, thumb reduplication, external ear malformations, and ventricular septal defect, in their patients. These clinical observations confirmed the variability of clinical manifestations in this group of patients, and led to the observation that a number of conditions that were previously considered unique conditions on a clinical basis are caused by mutations in the *SALL4* locus.

The most important condition known to overlap clinically with Okihiro syndrome is HOS, an autosomal dominant disorder characterized by radial ray malformations, congenital heart abnormalities, and associated features [Newbury-Ecob et al., 1996]. Although a major locus for HOS was identified at *TBX5*, only approximately 30% of the cases clinically identified as HOS were found to have a mutation at this locus [Brassington et al., 2003; Cross et al., 2000].

Other clinical conditions hypothesized to result from a mutation in the *SALL4* locus include AROS [Becker et al., 2002], and patients who were previously thought to have thalidomide embryopathy, but in whose offspring limb and cardiac malformations were also observed [McBride, 1994]. Indeed, Kohlhase et al. [2003] and Borozdin et al. [2004b] were able to identify a mutation in the *SALL4* gene in patients diagnosed with these conditions [Aalfs et al., 1996; Becker et al., 2002]. Furthermore, some *SALL4* mutation-positive patients had also been diagnosed as having either VACTERL association or TBS [Borozdin et al., 2004b; Kohlhase et al., 2003].

SALL4 consists of four coding exons (3,159 bp of coding sequence) [Deloukas et al., 2001; Kohlhase et al., 2002b]. The first exon carries the most likely initiator methionine and comprises 130 bp of coding sequence, followed by an intron of 9,926 kb in size. Exon 2 has a length of 2,331 bp and is separated from exon 3 by an intron of 880 bp. Exon 3 contains 281 bp coding sequence, followed by intron 3 of 4,176 bp and by exon 4, which consists of a 417 bp coding sequence and at least 1,952 bp of 3′UTR (GenBank Accession no. NM_020436.2, NT_011362.9). *SALL4* encodes three C2H2 double zinc finger domains of the SAL type, the second of which has a single C2H2 zinc finger attached at its carboxyterminal end, as well as an N-terminal C2HC zinc finger motif that is typical of vertebrate SAL-like proteins [Buck et al., 2000; Farrell and Munsterberg, 2000; Farrell et al., 2001; Hollemann et al., 1996; Kohlhase et al., 1996, 1999a, 2000, 2002a; Köster et al., 1997; Onuma et al., 1999; Ott et al., 1996]. A comparison of the amino acid sequences of *SALL1–3* with *SALL4* suggests that *SALL3* and *4* may have separated after the duplication of a common ancestor gene (data not shown). While *SALL3* encodes a fourth double zinc finger domain, which seems to be spliced out preferentially from the mRNA [Kohlhase et al., 1999a], the corresponding coding sequence is replaced by intron 2 in *SALL4*.

RT–PCR with primers positioned in exons 2 and 4, and exons 1 and 2 suggested that there is no alternative splicing in *SALL4*. However, GenBank sequences AY170621 (*SALL4B* mRNA) and AY170622 (*SALL4C* mRNA) suggest that two alternative splicing products exist in addition to the full-length A mRNA, the B mRNA containing 1,851 bp of coding sequence, and the C mRNA containing 831 bp. The B mRNA consists of exon 1, 1,150 bp of exons 2, 3, and 4, whereas in the C mRNA exon 2 is spliced out. The function of these splice products, which do not contain most of the zinc finger domains thought to be crucial for the function of *SALL* genes, has yet to be determined.

Mutations

Including the five novel mutations reported here for the first time, a total of 22 different *SALL4* mutations have been described (Table 1; numbering according to *SALL4* mRNA sequence (GenBank NM_020436.2)). These are five small duplications (c.496dupC (p.Q166fs), c.611_614dupCCGT (p.P206fs), c.941dupC (p.L315fs), c.1223_1226dupGACC (p.F410fs), and c.1835_1836dupAC (p.L613fs)), seven small deletions (c.326delC (p.P109fs), c.842delG (p.S281fs), c.1054delG (p.A352fs), c.1468-1471delTCTT (p.S490fs), c.1904delT (p.F635fs), c.2425delG (p.A809fs), and c.2477delC (p.P826fs)), two combined deletion/insertion mutations (c.899_900delCTinsG (p.P300fs) and c.2535_2536delTGinsA (p.F845fs)) and eight nonsense mutations (c.496C>T (p.Q166X), c.523A>T (p.K175X), c.1849C>T (p.R617X), c.1954C>T (p.Q652X), c.2288T>A (p.S763X), c.2491C>T (p.R831X), c.2593C>T (p.R865X), and c.2665G>T (p.E889X)). Seventeen of these are located in exon 2, and five are located within exon 3. No mutation was found in exon 1 or exon 4 [Al-Baradie et al., 2002; Borozdin et al., 2004b; Brassington et al., 2003; Kohlhase et al., 2002b, 2003].

Apart from point mutations or small deletions/duplications, larger deletions within or including the *SALL4* gene were recently found in six families with either Okihiro or AROS [Borozdin et al., 2004a]. Affected persons in two of those families carried heterozygous deletions including exons 1–3. In one, the deletion size was determined as being 59.193 kb with breakpoints within Alu elements (J. Kohlhase, unpublished data), and in the other family it was 35–65 kb, with the exact breakpoints still unknown. In one family, patients were heterozygous for a deletion including exon 4 but no other parts of the coding region, and in another family a deletion of 8.888 kb was found, including exon 1 as the only part of the coding region. Only one of the two breakpoints of the latter deletion is situated within an Alu element. These

TABLE 1. SALL4 Mutations*

Base change	Amino acid change	Position	Phenotype	Reference
c.326delC	p.P109fs	Exon 2	Thalidomid-Oki	Kohlhase et al. [2003]
c.496C>T	p.Q166X	Exon 2	Oki/ HOS	Borozdin et al. [2004b]
c.496dupC	p.Q166fs	**Exon 2**	**Oki**	**This report**
c.523A>T	p.K175X	Exon 2	Oki	Kohlhase et al. [2003]
c.611_614dupCCGT[a]	p.P206fs	Exon 2	Oki	Brassington et al. [2003]
c.842delG	p.S281fs	Exon 2	Oki	Kohlhase et al. [2002b]
c.899_900delCTinsG	p.P300fs	Exon 2	Oki/ AROS	Borozdin et al. [2004b]
c.941dupC*	p.L315fs	Exon 2	Oki	Kohlhase et al. [2002b]
c.1054delG[a]	p.A352fs	Exon 2	Oki	Kohlhase et al. [2002b]
c.1223_1226dupGACC	**p.F410fs**	**Exon 2**	**Oki/ AROS**	**This report**
c.1468_1471delTCTT	**p.S490fs**	**Exon 2**	**Oki**	**This report**
c.1835_1836dupAC	**p.L613fs**	**Exon 2**	**Oki**	**This report**
c.1849C>T	p.R617X	Exon 2	Oki	Kohlhase et al. [2003]
c.1904delT	p.F635fs	Exon 2	Oki	Al-Baradie et al. [2002]
c.1954C>T	p.Q652X	Exon 2	Oki	Kohlhase et al. [2002b]
c.2288C>A	p.S763X	Exon 2	Oki	Kohlhase et al. [2002b]
c.2425delG	p.A809fs	Exon 2	Oki	Al-Baradie et al. [2002]
c.2477delC	p.P826fs	Exon 3	AROS	Borozdin et al. [2004b]
c.2491C>T	p.R831X	Exon 3	Oki	Borozdin et al. [2004b]
c.2535_2536delTGinsA	**p.F845fs**	**Exon 3**	**Oki**	**This report**
c.2593C>T	p.R865X	Exon 3	Oki/ AROS	Al-Baradie et al. [2002]; Kohlhase et al. [2003]
c.2665G>T	p.E889X	Exon 3	Oki/AROS	Borozdin et al. [2004b]

*Mutations reported here for the first time were detected by direct sequencing analysis of *SALL4* PCR products as described [Kohlhase et al., 2002b] and confirmed by sequencing of a second PCR product and segregation analysis in families. Numbering according to *SALL4* mRNA sequence (GenBank NM_020436.2). Novel mutation data (bold) were reviewed but are not shown.
[a]Numbering was changed from original reports according to mutation nomenclature rules.

deletions occurred de novo in two sporadic cases, and segregated with the phenotype in the remaining families.

Up to now, it has not been possible to correlate the severity of the phenotype with the mutation position. The strongest argument is that the mutation c.2593C>T may cause either a mild or a severe phenotype [Kohlhase et al., 2003]. In view of the deletions of the complete coding region causing Okihiro syndrome with no obvious difference in the phenotype caused by mutations, it seems that the point mutations and smaller deletions/ duplications also result in haploinsufficiency (see below). The positions of the mutations and deletions with respect to the SALL4 protein are shown in Figure 1.

The detection rate of *SALL4* mutations in patients with Okihiro syndrome was 100% in the report of Al-Baradie et al. [2002], and 62.5% (5/8) in the first report of Kohlhase et al. [2002b]. The remaining three of eight families with Okihiro syndrome were found to have larger deletions [Borozdin et al., 2004a]. There are no data yet to suggest locus heterogeneity for Okihiro syndrome, although in some patients with Okihiro syndrome no mutations or larger deletions have been found (J. Kohlhase, unpublished data). However, current methods of mutation detection do not cover intron or promoter sequences. Therefore, the question of genetic heterogeneity requires more comprehensive gene testing before it can be settled.

No pathogenic missense mutations in *SALL4* have been reported to date. Based on the current knowledge of *SALL4*, only missense mutations resulting in haploinsufficiency would be expected to cause Okihiro syndrome. Therefore, missense mutations in *SALL4* might be found in Okihiro patients only at positions that are essential for *SALL4* function. In the current absence of functional studies, only the replacement of amino acids located within the zinc finger domains could be expected to be deleterious. As was previously shown for the transcription factor *Krüppel* in *Drosophila melanogaster*, replacement of an essential cysteine residue within the second of five tandemly arranged zinc finger motifs leads to a functional null allele [Redemann et al., 1988]. It is therefore possible that similar missense mutations of *SALL4* will be detected as the number of analyzed patients increases. However, the question remains as to whether missense mutations within other parts of *SALL4* would result in a phenotype different from Okihiro syndrome.

Nonpathogenic Variants

Fifteen nonpathogenic variants have been identified in patients and unaffected parents screened for *SALL4* mutations (Table 2; numbering according to *SALL4* mRNA sequence (GenBank NM_020436.2)), eight of which are reported here for the first time (c.352G>A (p.A118T), c.588G>A (p.R196R), c.615G>T (p.V205V), c.645C>T (p.L215L), c.795T>C (p.S265S), c.1056G>A (p.A352A), c.3099T>C (p.T1033T), and c.3287T>A (3′UTR)). No clear estimate of the occurrence rates of these sequence variations in normal populations has been given, but the most common (in >10% of analyzed patients; Kohlhase et al., unpublished results) seem to be c.645C>T, c.1056G>A, c.1520T>G, c.1860A>G, c.2037C>T, c.2392A>C (all exon 2), and c.2640G>C (exon 3). The high prevalence of heterozygous SNPs in the *SALL4* coding sequence is very helpful in identifying disease-causing deletions (Borozdin et al., 2004a]. At position 540 of the cDNA, the "variant" c.540C (p.N180N) [Al-Baradie et al., 2002] was found homozygously in all but one sample (out of more than 150) examined by the corresponding author's group, and this other sample was heterozygous for 540T, indicating that the c.540T allele most likely represents a rare variant. The variant c.2260G>A (p.V754M) was reported (under the wrong designation of p.V752M) as a disease-causing mutation [Brassington et al., 2003], but is listed here as a (likely) nonpathogenic variant given the lack of evidence for its pathogenicity. This change does not affect any known functional

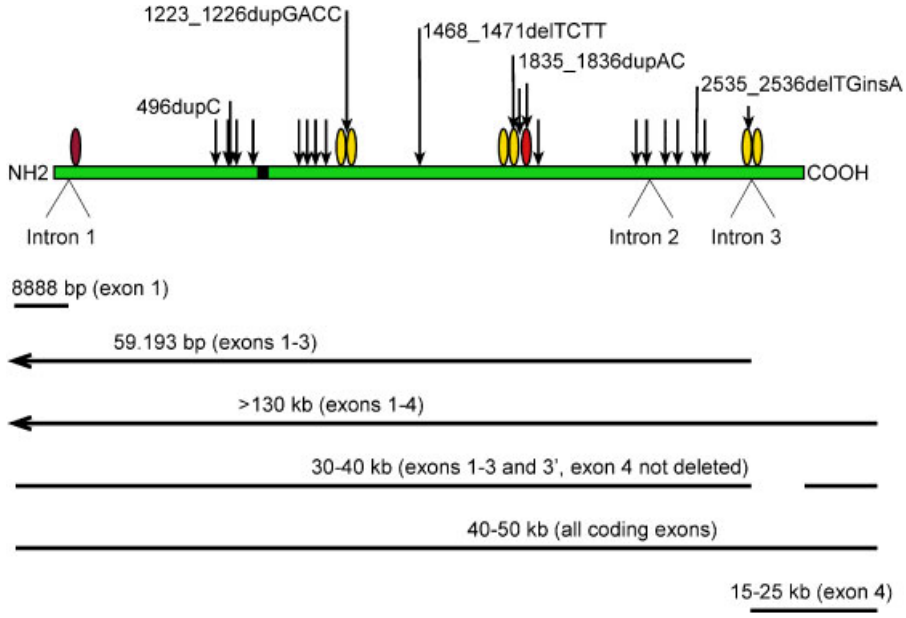

FIGURE 1. **Schematic representation of the *SALL4* protein and localization of the mutations and deletions identified to date. Zinc fingers are indicated as oval symbols. *SALL4* encodes three C2H2 double zinc finger domains distributed over the protein. A single C2H2 domain is attached to the second double zinc finger. At the aminoterminus, a single C2HC domain is found. Horizontal bars indicate the positions of bigger deletions with respect to the coding exons. The interrupted bar indicates that this deletion spares exon 4 but continues farther 3′. Note that the mutations c.496 dupC and c.2593C>T have each been found in two unrelated families. All other mutations have been found only once. The newly described mutations are labeled, and the unlabeled arrows represent previously described mutations. Positions of the introns are indicated. Numbers refer to the amino acid sequence (1053 aa). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]**

TABLE 2. **SALL4 Nonpathogenic Variants***

Base change	Amino acid change	Position	Reference
c.352G>A	**p.A118T**	**Exon 2**	**This report**
c.540T>C	p.N180N	Exon 2	Al-Baradie et al. [2002]
c.588G>A	**p.R196R**	**Exon 2**	**This report**
c.615G>T	**p.V205V**	**Exon 2**	**This report**
c.645C>T	**p.L215L**	**Exon 2**	**This report**
c.795T>C	**p.S265S**	**Exon 2**	**This report**
c.1056G>A	**p.A352A**	**Exon 2**	**This report**
c.1520T>G	p.L507R	Exon 2	Al-Baradie et al. [2002]
c.1860A>G	p.T620T	Exon 2	Al-Baradie et al. [2002]
c.2037C>T	p.T679T	Exon 2	Al-Baradie et al. [2002]
c.2260G>A[a]	p.V754M	Exon 2	Brassington et al. [2003]
c.2392A>C	p.I798L	Exon 2	Al-Baradie et al. [2002]
c.2640G>C	p.S880S	Exon 3	Al-Baradie et al. [2002]
c.3099T>C	**p.T1033T**	**Exon 4**	**This report**
c.3287T>A	**3'UTR**	**Exon 4**	**This report**

*Nonpathogenic variants reported here for the first time (bold) were detected by direct sequencing analysis of *SALL4* PCR products of patients as described elsewhere [Kohlhase et al., 2002b]. Novel variants were confirmed by sequencing of a second PCR product. Segregation analysis in families showed that they did not segregate with the phenotype. Numbering according to *SALL4* mRNA sequence (GenBank NM_020436.2).
[a]c.2260G>A was reported (under the wrong designation V752M) as disease-causing, but is listed here as a variant due to lack of evidence for pathogenicity since it neither affects any known functional domain of SALL4 nor was it shown to segregate with the phenotype [Brassington et al. 2003].

domain of SALL4, nor has it been shown to segregate with the phenotype.

Biological Relevance

All *SALL4* mutations detected to date produce preterminal stop codons (Table 1). Since transcripts carrying a preterminal stop codon, which is separated by at least one intron from the last exon, are mostly rapidly degraded, all known mutations are likely to be disease-causing via SALL4 haploinsufficiency [Maquat, 2004]. This interpretation is supported by the observation of disease-causing deletions of all coding exons in patients from two independent families that clearly resulted in haploinsufficiency [Borozdin et al., 2004a]. An earlier indication for SALL4 haploinsufficiency as the cause for Okihiro syndrome and AROS was the observation of similar malformations in two patients with a deletion of the chromosomal region including the *SALL4* gene [Fraisse et al., 1981; Shabtai et al., 1993]. However, because of the small number of analyzed patients, it remains unclear whether point mutations and smaller deletions/insertions result in

a different phenotype compared to that caused by larger deletions. If they do, one would have to speculate that there is an additional dominant (-negative) action of the mutated alleles similar to the one presumed for *SALL1* mutations [McLeskey Kiefer et al., 2003].

In a study by Kohlhase et al. [2002b], expression of *SALL4* in adult human tissue was seen only in the testis and ovary, and not in the brain, heart, skeletal muscle, lung, liver, or spleen, by northern blot and RT-PCR. EST data at UniGene suggest further expression in skin, small intestine, muscle, brain, and embryonic stem cells. No data on the developmental expression in humans are available. In the mouse, *Sall4* mRNA expression [Kohlhase et al., 2002a] was detected by whole-mount in situ hybridization as early as 7.5 days postcoitus (d.p.c.) and appeared widespread in the embryo. At 8.5 d.p.c., *Sall4* expression was seen in the head region and the primitive streak. In 9.25 d.p.c. embryos, *Sall4* expression was strong in the tail bud/presomitic mesoderm, and upregulated in the midbrain, mandibular arch, and frontonasal mesenchyme. At 10.5 d.p.c., additional *Sall4* expression was seen in the forelimb bud mesenchyme and the maxilla. At 11.5 d.p.c., *Sall4* expression appeared graded in several embryonic regions, i.e., in the midbrain (strongest at the isthmus), posterior trunk region, limb bud mesenchyme, and genital papilla. At 12.5 d.p.c., *Sall4* expression was maintained only in the posterior trunk region, with a ring of stronger expression in the presomitic mesoderm.

The expression in the progress zone of the limb buds fits well with the radial ray anomalies in patients with *SALL4* mutations. A reduced amount of human *SALL4* transcript in the mesenchyme of the progress zone could explain why limb outgrowth is inhibited in patients who may show severe limb reduction. A reduced dosage of *SALL4* in the mid-hindbrain region and the branchial arches may result in disturbed development of the VIth cranial nerve and/or its nucleus and hearing loss, respectively, as described in Okihiro syndrome. It remains to be elucidated whether the expression of *Sall4* and *SALL4* [Kohlhase et al., 2002a,b] in the testis and ovary reflects an additional function of these genes for germ cell development.

Clinical and Diagnostic Relevance

As with *SALL1*, there is significant intra- and interfamilial variability in the clinical presentation of patients with *SALL4* mutations. This is especially evident by diagnoses made in patients who were later found to have a *SALL4* mutation. Among these are AROS (which is now thought to be allelic with Okihiro syndrome), TBS (which is sometimes confused with Okihiro syndrome in patients who do not have radial hypoplasia or aplasia, but do have triphalangeal thumbs and/or preaxial polydactyly), VACTERL association, HOS, or thalidomide embryopathy [Brassington et al., 2003; Borozdin et al., 2004b; Kohlhase et al., 2002b, 2003; Kohlhase and Holmes, 2004]. Thus, *SALL4* mutation analysis can be helpful in finding the cause of malformation syndromes seen in children in whom *SALL1* (TBS) or *TBX5* tests fail to show a mutation. When should a *SALL4* test be considered? Current data suggest that a Duane anomaly is present in less than 70% of *SALL4* mutation/deletion carriers, so a Duane anomaly is not fully required [Kohlhase, 2004]. At present, it seems that a combination of radial ray defects with a Duane anomaly is a clear indication for *SALL4* testing, as long as other causes, such as Fanconi anemia and chromosomal aberrations, are excluded.

When HOS is considered, the presence of renal or ear malformations, or hearing loss, in addition to the typical features point toward *SALL4*. It seems that most of the patients who carried the initial diagnosis of HOS and were found to have a *SALL4* mutation did not have true HOS, if the presence of only radial and heart defects (but no other malformations) is considered to allow the diagnosis of HOS [Brassington et al., 2003; Borozdin et al., 2004b; Kohlhase et al., 2003]. However, most of those patients did not have a Duane anomaly or structural eye defects, and thus were also not typical for Okihiro syndrome or AROS. The severity of a heart defect is not helpful for differentiating between Okihiro syndrome and HOS because complex malformations (e.g., Fallot's tetralogy) can also be associated with a *SALL4* mutation [Borozdin et al., 2004b]. When VACTERL is considered, current observations suggest that the presence of tracheoesophageal fistulas excludes *SALL4* mutations; however, the data are limited at present (Kohlhase, unpublished data). Occasionally, TBS is diagnosed based on the combination of imperforate anus and dysplastic ears with radial hypo- or aplasia and thumb aplasia. These severe radial malformations appear to exclude a *SALL1* mutation and the diagnosis of TBS (Kohlhase, unpublished data), but do indicate a *SALL4* defect.

To date, mutation analyses of *SALL4* have failed to demonstrate a clear genotype–phenotype correlation. There is no obvious correlation between the clinical presentation and the nature or location of specific point mutations. Currently, one out of two mutations reported in two families is c.2593C>T [Al-Baradie et al., 2002; Kohlhase et al., 2003]. This mutation may result in either severe or mild phenotypes. The mutation c.496dupC (reported here) has also been found in two independent families (in one Polish family it was inherited over at least two generations, and in the other family it was found in a sporadic case). With the latter mutation, the phenotype appears to be similar between the different families (Table 3). Since each of the other mutations was found in only one family, it remains unclear whether the different phenotypes are caused by additional different effects of the mutation, or are more likely due to epigenetic influences or to the effect of as-yet-unknown modifier genes. Nevertheless, the CG dimer of nucleotides 2593–2594 may represent a true mutational hotspot that is prone to deamination of a 5′ methylcytosine. The position 496 may also be prone to duplications/deletions due to slippage of the DNA polymerase at the stretch of nucleotides 484–496, which consists of 11 cytosines interrupted by two adenines.

Currently, it seems unlikely that the mutation position with respect to the three different splice isoforms of *SALL4* has an effect on the phenotype, since mutations within the 3′ part of exon 2, which is missing in isoform B, can cause milder or severe phenotypes. This is similar to the case of mutations within exon 3, which is present in all three isoforms. Higher patient numbers are required, however, to substantiate this observation.

Prior to the identification of *SALL4* mutations as the cause of Okihiro syndrome, it was reported that penetrance was not complete [Hayes et al., 1985]. Molecular data confirmed this for a second family [Collins et al., 1993; Kohlhase et al., 2002b], but are not yet available for the family reported by Hayes et al. [1985]. In the family reported by Collins et al. [1993], the phenotype (especially regarding radial ray abnormalities) is mild in all family members who carry the mutation. It seems therefore that reduced penetrance may only be expected when the phenotype is very mild in at least one family member. This hypothesis would also fit with the family reported by Hayes; however, further data are required to support this assumption. In all other families analyzed for *SALL4* mutations, penetrance was complete. Other than in TBS, no case of *SALL4* germline mosaicism has been observed.

TABLE 3. Phenotypic Features of Family Members With Newly Described Mutations*

Patient	Eyes	Arms	Ears	Hearing	Kidneys	Feet	Heart	Other
Family 1								
Mother	N	Bil. TT, HPLTH, left arm 1 cm shorter	N	N	NR	N	NR	Choanal atresia
Daughter	Bil. DA	HPLT R, TT L, Radial deviation of hands	N	N	NR	NR	NR	
Family 2								
Daughter (sporadic)	DA R	HPLTH L + PPD R	N	Mild CHL	HSK	NR	NR	Submucous cleft palate, choanal atresia L, short stature
Family 3								
Mother	DA L, IC L, RC L	TT R	N	N	N	N	N	Short stature
Daughter	IC L, RC L	Bil. TT	N	N	N	N	N	Hip dislocation, short stature, hypophyseal hypoplasia
Family 4								
Son (sporadic)	Bil. DA	TT R, HPLT L, SD I-II L, AMC1 L	N	N	Pelvic kidney	NR	N	Asymmetric claviculae with hook L, Bil. epic., hypertelorism, 11 rib pairs
Family 5								
Mother	Bil. DA	Bil. HPLT + AMC1	NR	NR	NR	N	N	
Fetus 1	NR	HPLR + HPLU + CH L (ultrasound)	NR	NR	NR	NR	NR	Pregnancy terminated
Fetus 2	NR	Radial ray defects (ultrasound)	NR	NR	NR	NR	NR	Pregnancy terminated
Family 6								
Daughter (sporadic)	Bil. DA	Bil. AR, Bil. AT, HPLH R > L	N	N	Low set kidneys, R crossing midline	N	PDA	Mild scoliosis, SPO, leg length R < L, Bil. femoral head HPL

*Mutations: Families 1 and 2: c.496dupC, Family 3: c.1223_1226dupGACC, Family 4: c.1468_1471delTCTT, Family 5: c.1835_1836dupAC, Family 6: c.2535_2536delTGinsA.

AMC1, absent metacarpal 1; AR, absent radius/radii; AT, absent thumb(s); Bil, bilateral; CH, clubbed hand; CHL, conductive hearing loss; DA, Duane anomaly; dilat., dilatation; disloc., dislocated; epic., epicanthic folds; HL, Hearing loss; HN, hydronephrosis; HPL, hypoplasia; HPLH, humerus hypoplasia; HPLK, hypoplastic kidney; HPLR, radius hypoplasia; HPLU, ulna hypoplasia; HPLT, thumb hypoplasia; HPLTH, thenar hypoplasia; HSK, horseshoe kidney; IC, iris coloboma; L, left; N, normal; NR, no abnormality reported; PC, pelvicalyceal; PDA, patent ductus arteriosus; PPD, preaxial polydactyly; PSS, prenatal serum screening; R, right; RC, retinal coloboma; SD, Syndactyly; SNHL, sensorineural hearing loss; SPO, spina bifida occulta; TT, triphalangeal thumb.

Future Prospects

The full spectrum of *SALL4* mutations in patients with Okihiro syndrome and overlapping phenotypes is still being delineated. It will be interesting to see if missense mutations at different positions of *SALL4* also cause Okihiro syndrome or other phenotypes. The identification of such mutations may also help to identify essential functional domains of the SALL4 protein other than the zinc fingers. Also, extensive functional studies on *SALL4* including animal models are required to explain why loss of SALL4 function leads to the observed malformations. However, the observation that the *Sall1* knockout in the mouse had a phenotype dissimilar to TBS [Nishinakamura et al., 2001] may indicate that there are differences between mouse and human *SALL* gene functions, and that caution should be used when interpreting a future *Sall4* mouse model.

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