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Nonrecurrent *PMP22-RAI1* contiguous gene deletions arise from replication-based mechanisms and result in Smith-Magenis syndrome with evident peripheral neuropathy

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Abstract

Hereditary neuropathy with liability to pressure palsies (HNPP) and Smith-Magenis syndrome (SMS) are genomic disorders associated with deletion copy number variants involving chromosome 17p12 and 17p11.2, respectively. Nonallelic homologous recombination (NAHR)-mediated recurrent deletions are responsible for the majority of HNPP and SMS cases; the rearrangement products encompass the key dosage sensitive genes *PMP22* and *RAI1*, respectively, and result in haploinsufficiency for these genes. Less frequently, nonrecurrent genomic rearrangements occur at this locus. Contiguous gene duplications encompassing both *PMP22* and *RAI1*, i.e. *PMP22-RAI1* duplications, have been investigated, and replication-based mechanisms rather than NAHR have been proposed for these rearrangements. In the current study, we report molecular and clinical characterizations of six subjects with the reciprocal phenomenon of deletions spanning both genes, i.e. *PMP22-RAI1* deletions. Molecular studies utilizing high-resolution array comparative genomic hybridization and breakpoint junction sequencing identified mutational signatures that were suggestive of replication-based mechanisms. Systematic clinical

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Conflicts of interest statement

J.R.L. has stock ownership in 23andMe, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen Inc., is a member of the Scientific Advisory Board of Baylor Miraca Genetics Laboratories, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from the chromosomal microarray analysis (CMA) and clinical exome sequencing offered in the Baylor Miraca Genetics Laboratory.

studies revealed features consistent with SMS, including features of intellectual disability, speech and gross motor delays, behavioral problems and ocular abnormalities. Five out of six subjects presented clinical signs and/or objective electrophysiologic studies of peripheral neuropathy. Clinical profiling may improve the clinical management of this unique group of subjects, as the peripheral neuropathy can be more severe or of earlier onset as compared to SMS patients having the common recurrent deletion. Moreover, the current study, in combination with the previous report of *PMP22-RAI1* duplications, contributes to the understanding of rare complex phenotypes involving multiple dosage-sensitive genes from a genetic mechanistic standpoint.

Keywords

Contiguous gene deletion; *PMP22*; *RAI1*; FoSTeS/MMBIR

Introduction

DNA rearrangements have been demonstrated to cause gene interruption or fusion by disrupting gene structure, altering the copy number of dosage-sensitive gene(s), or imposing a position effect on the regulation of gene expression; the latter is potentially mediated through altering Topologically Associating Domains (TAD) or effecting long noncoding RNA (lncRNA) (Lupianez et al. 2015; Szafranski et al. 2016). Deletion of a genomic segment may unmask recessive mutation(s) on the other allele (Liburd et al. 2001; Wu et al. 2015). Collectively, diseases mediated by such DNA rearrangements are termed genomic disorders (Lupski 1998, 2009, 2015; Lupski and Stankiewicz 2005).

Copy number variants (CNVs), including duplications or deletions of a genomic segment on the proximal short arm of human chromosome 17, have been associated with various genomic disorders. Interstitial deletion CNVs on 17p11.2 encompassing *RAI1* result in Smith-Magenis syndrome (SMS, MIM: 182290); and a recurrent deletion of ~3.7 Mb in size is observed in 70-80% of all deletion cases. SMS is a disorder characterized by distinctive physical features including a recognizable pattern of craniofacial dysmorphism, obesity, developmental delay, cognitive impairment, and neurobehavioral abnormalities. Infants often have feeding difficulties, failure to thrive, hypotonia and hyporeflexia. Variable manifestations include congenital heart defects, hearing loss, renal anomalies, scoliosis, short stature, and progressive myopia. The behavioral characteristics include significant sleep disturbance, stereotypies, and selfinjurious behaviors, which usually appear after 18 months and continue through adulthood (Neira-Fresneda and Potocki 2015). Although haploinsufficiency of *RAI1* caused by loss of function mutations has been reported as the primary cause for most of the SMS features (Bi et al. 2006; Girirajan et al. 2006; Slager et al. 2003), phenotype-genotype correlation studies in humans and chromosome-engineered mouse CNV models have proposed that other genes in or beyond the 17p11.2 deletion region may undergo dosage imbalance or perturbed regulation due to the deletion and as a result contribute to the variable expressivity of the syndrome (Bi et al. 2002; Girirajan et al. 2006; Lacaria et al. 2012; Ricard et al. 2010; Yan et al. 2007).

In a nearby genomic region, a recurrent interstitial deletion of ~1.4 Mb in size on chromosome band 17p12 encompassing *PMP22* is associated with hereditary neuropathy with liability to pressure palsies (HNPP, MIM: 162500) (Chance et al. 1993). Truncating mutations in *PMP22* have also been found in subjects with HNPP phenotypes indistinguishable from those with the 1.4 Mb common HNPP deletion (Li et al. 2007). HNPP is characterized by recurrent entrapment neuropathies such as peroneal palsy with foot drop and carpal tunnel syndrome (Potocki et al. 1999). The disorder usually presents during the second or third decade, even though it could present atypically in early childhood (Chrestian et al. 2015). The electrophysiological pattern of HNPP can potentially be quite characteristic with uniform demyelinating sensory-motor polyneuropathy and typical multifocal conduction slowing at sites of entrapment; i.e. conduction block (CB) (Gabreels-Festen et al. 1992). *PMP22* haploinsufficiency may hasten the induction of CB and thus protein product encoded by *PMP22* appears to protect the nerve from mechanical injury (Bai et al. 2010).

The SMS deletion affects the central nervous system (CNS), leading to intellectual disability and behavior abnormalities accompanied by developmental delay and distinctive facial features. The deletion may also have peripheral nervous system (PNS) involvement and manifest signs suggestive of peripheral neuropathies, but with normal nerve conduction velocities (NCVs) in most SMS cases (Greenberg et al. 1991). HNPP is a mild form of peripheral neuropathy, which may be underdiagnosed due to episodic and transient clinical manifestations (Potocki et al. 1999). HNPP disease expression, including electrophysiologic manifestations, can be influenced significantly by environmental exposures – as noted in the initial clinical description by “a liability to pressure palsies”, mechanical stress to peripheral nerves can elicit a pressure palsy (De Jong 1947; Staal et al. 1965). However, when both *PMP22* and *RAI1* are deleted it is plausible to hypothesize that the interplay between CNS and PNS abnormalities may lead to a variable, potentially more severe phenotype blending both SMS and HNPP.

The recurrent SMS and HNPP deletions are generated by nonallelic homologous recombination (NAHR) using flanking low copy repeats (LCRs) as substrates, termed SMS-REPs and CMT1A-REPs, respectively (Chen et al. 1997; Pentao et al. 1992). These long and highly homologous sequences promote unequal crossover and subsequently result in copy number changes (Dittwald et al. 2013; Liu et al. 2011; Yuan et al. 2015b). However, since the SMS and HNPP recurrent deletions are delimited by distinct LCR pairs, extensive homologies flanking both SMS and HNPP critical regions as a contiguous segment are lacking.

Interestingly, there have been reports of rare large deletions that encompass both HNPP and SMS loci and include both *PMP22* and *RAI1* (Goh et al. 2014; Juyal et al. 1996; Liu et al. 2011; Stankiewicz et al. 2003; Trask et al. 1996; Zori et al. 1993). These deletions have breakpoints that are potentially beyond the regions defined by the LCR-mediated NAHR; thus, a different mechanism may be considered and investigated. Moreover, because different spectrums of gene contents are involved in these large deletions, we provided a comprehensive documentation of the clinical profiles of the subjects with *PMP22-RAI1* contiguous gene deletions. The study of *PMP22-RAI1* deletions (N = 6 reported herein)

complements our previous report of *PMP22-RAII* duplications (N = 23, Yuan et al. 2015a), both from the molecular and clinical perspectives.

Subjects and Methods

Human subjects

A total number of 132 subjects with chromosome 17p11.2 deletion were enrolled in a research protocol approved by the Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals. Informed consent was obtained from the subjects and parents or legal guardians of the subjects. All genomic studies, including array CGH and breakpoint junction sequencing, were performed on DNA samples extracted from whole blood.

Six of 132 subjects were identified with larger deletions encompassing both *PMP22* and *RAII*, deletions of which are responsible for HNPP and SMS, respectively. BABs 484, 608, 993, and 2011 were published with molecular mapping and/or clinical findings (Juyal et al. 1996; Liu et al. 2011; Stankiewicz et al. 2003; Zori et al. 1993); among these, breakpoint junction sequences was previously obtained for BAB2011 (Liu et al. 2011). BABs 8499 (also known as SMS530) and 8501 were not reported previously. Detailed clinical records were available from 6/6 subjects for review. Detailed clinical information is listed in Supplemental Table 1.

Array comparative genomic hybridization (aCGH) and breakpoint junction sequencing

aCGH with high density coverage of the proximal 17p region was designed to interrogate CNVs with high resolution. Array design (AMADID# 032121), experimental procedures, aCGH data analysis, and breakpoint junction sequencing methods were as described (Yuan et al. 2015a). Breakpoint junction sequences were mapped to hg19.

Mosaicism quantification by aCGH

The mosaicism level of deletion on autosomes (α) was calculated based on aCGH $\log_2$ ratio (LR), which was the mean LR of all probes involved in the genomic segments that were deleted:

$$\alpha = \frac{1 - 2^{LR}}{0.5}$$

Results

We characterized deletion CNVs overlapping chromosome 17p11.2 in 132 cases with a molecular and clinical diagnosis of SMS. NAHR mediated common recurrent SMS deletion (CR) was identified in 96 (72.7%) cases; uncommon recurrent deletions 1, 2 and 3 (UR1, UR2 and UR3) were identified in eight (6.0%), two (1.5%) and two (1.5%) cases, respectively (Fig. 1). We identified nonrecurrent deletions (NR) with unique CNV boundaries nonetheless spanning *RAII* in 24 (18.2%) subjects. Among these NR deletions, we identified six (6/24, 25.0%) subjects with deletions encompassing both *PMP22* and

RAI1, termed *PMP22-RAI1* contiguous gene deletion, or in short *PMP22-RAI1* deletion (Fig. 1b, in red). These deletions range from 4.6 Mb to 9.4 Mb, with the smallest region of overlap (SRO) of ~2.8 Mb spanning *PMP22* and *RAI1* in one genomic segment (Fig. 2a). No other submicroscopic CNVs were observed in these six cases based on genome-wide chromosome microarray analysis (**CMA data not shown**).

Breakpoint junction sequencing was attempted for the *PMP22-RAI1* deletions in these six subjects in order to map the CNV boundaries to nucleotide-resolution and surmise potential molecular mechanisms. Microhomologies of different lengths were identified at the breakpoint junctions of BAB2011 (1 bp, C) and BAB8499 (6 bp, ACCACC) (Fig. 2b, Table 1), consistent with fork stalling and template switching/microhomology-mediated break-induced replication (FoSTeS/MMBIR) (Hastings et al. 2009; Lee et al. 2007; Zhang et al. 2009). Microhomology-mediated end-joining (MMEJ) may be an alternative explanation, as microhomologies were identified at the breakpoint junction (McVey and Lee 2008).

We were not able to experimentally determine the precise breakpoint junctions for BABs 484, 608, 993 and 8501, because the deletions in these cases had one boundary located in regions clustered with LCRs (17p-PROX for BABs 484, 608 and 993, *RNU3* LCRs for BAB8501, Fig. 1), limiting the ability to uniquely define the locus of the breakpoint junction. It has been proposed that complex genomic architectures may catalyze nonrecurrent genomic rearrangement and cluster their breakpoints in the vicinity of LCRs (Carvalho and Lupski 2016; Stankiewicz and Lupski 2010). The 17p proximal region is enriched with LCRs, which constitute higher-order complex features of this region and thereby promote genomic instability and mediate recurrent (NAHR) and stimulate nonrecurrent (via template switching) rearrangement events that lead to CNVs (Stankiewicz et al. 2003; Yuan et al. 2015a).

Using high-resolution aCGH data, we were able to re-analyze the CNV boundaries and estimate if they were embedded in regions of LCRs. In the cohort of 132 subjects identified with 17p11.2 deletions, LCR-mediated NAHR are responsible for the recurrent deletions including 96 CR, eight UR1, two UR2 and two UR3. For the remaining 24 NR deletions, at least 14/24 (BABs 608, 484, 8501, 993, 572, 1140, 641, 1221, 1195, 255, 1931, 1774, 566, 1615) had one boundary localized within regions of LCRs (Fig. 1). Among these, 6/14 (BABs 1140, 255, 1931, 1774, 566 and 1615) had one boundary inside the proximal SMS-REP; while 3/14 (BABs 608, 484 and 993) had one boundary inside the 17p-PROX region, which is composed of LCRs sequences flanking an ~141 kb stretch of microsatellite DNA sequences possibly responsible for the nonrecurrent rearrangements identified in the 17p proximal region (Yuan et al. 2015a).

We performed phenotype-genotype correlation studies to specifically describe the clinical profiles of the six subjects harboring the *PMP22-RAI1* deletion. Clinical features are summarized as Table 2. In general, among these subjects, the age of the latest evaluation was variable, ranging from 2 years to mid-20's. The most common features shared among these subjects were intellectual disability, speech and gross motor delays, behavioral problems and ocular abnormalities, which were consistent with features of SMS.

We further studied the severity of peripheral neuropathy as well as a potential spectrum of phenotypes broader than those usually observed in either SMS or HNPP in these subjects. We also compared the clinical features of these subjects with the reported SMS features from published genotype-phenotype correlation studies (Edelman et al. 2007; Girirajan et al. 2006; Greenberg et al. 1991; Greenberg et al. 1996) (Supplemental Tables 1 and 2). Two of six subjects (BABs 484 and 608) had a diagnosis of peripheral neuropathy. BAB484, who harbors an 8.0 Mb deletion, had decreased deep tendon reflexes (DTRs) along with a moderate motor slowing (MCV 23.9 m/sec) of both proximal median nerves with no sensory abnormalities on nerve conduction studies (NCS) at the age of 28 months. At 47 months her nerve conduction studies were reportedly within normal limits. Later in life at about 6 years of age she was given a clinical diagnosis of HNPP based on her symptoms, as well as prediction from her cytogenetic results. BAB608, whose deletion of 9.4 Mb was the largest among the subjects of this study, presented on clinical evaluation with motor delay, delayed walking and normal DTRs. This same subject had nerve conduction studies that showed a generalized peripheral nerve involvement including abnormalities of motor and sensory conduction of both lower extremities, suggesting Charcot-Marie-Tooth disease (CMT) but was later diagnosed with HNPP. One additional subject (BAB993), who has a 6.9 Mb deletion, presented on exam with *pes planus*, normal deep tendon reflexes, and abnormal gait, along with slowing in motor conduction velocities in both median nerves. The remaining subjects analyzed did not have NCS performed, but among these remaining individuals two additional subjects (BABs 2011 and 8499) were reported with abnormal gait, frequent falling, foot deformities and decreased sensitivity to pain, requirement of shoe inserts and orthotic interventions. Age of walking for these six subjects ranged from 18-36 months. One subject (BAB8501) did not present any readily apparent clinical signs of peripheral neuropathy at the most recent examination at age 23 years.

Few other congenital anomalies were reported among these subjects. Ophthalmologic abnormalities were by far the most common including myopia, strabismus, iris abnormalities and retinal detachment. Congenital heart malformation was reported in two out of six subjects (BABs 484 and 608). No renal abnormalities were identified within the chart review. BAB484 was found to have moderate hydrocephalus on brain MRI, involving lateral, third and fourth ventricles. BAB993 had prominence of the ventricular system, as previously reported in 9/25 patients with SMS (Greenberg et al. 1996).

In our study, we also used aCGH to re-evaluated the DNA isolated from the blood sample of BAB485, the mother of BAB484, who was reported in the past to have a mosaic *PMP22-RAI1* deletion characterized by karyotyping (Zori et al. 1993). The mosaicism level was quantified to be 57% by aCGH, which was consistent with the quantification by karyotyping (Zori et al. 1993). The clinical features of BAB485 are not consistent with SMS, but she did have some facial dysmorphism as well as *pes cavus* but normal DTRs. Her nerve conduction studies did not show any evidence of peripheral neuropathy. In contrast to BAB485, one mosaic case for the *PMP22-RAI1* deletion reported by Goh *et al* (Goh et al. 2014) did have facial features and developmental delays consistent with SMS, as well as hyporeflexia. NCS studies at the age of 7.5 years confirmed polyneuropathy. However, NCS values were not provided in the report.

Discussion

Genomic architecture with features of LCRs/segmental duplications and repetitive sequences (such as *Alus*) may predispose to rearrangements. In addition to providing extensive homology for NAHR, LCRs may contribute to genomic instability and template switches leading to nonrecurrent rearrangements with breakpoints grouping at LCR regions via mechanisms other than NAHR (Carvalho and Lupski 2016; Stankiewicz and Lupski 2010; Stankiewicz et al. 2003; Yuan et al. 2015a). In fact, breakpoint junctions have been sequenced in several of the deletions with one boundary in LCRs, and microhomology or small insertions were revealed (Shaw and Lupski 2005). The evidence suggests that replication-based mechanism or non-homologous end joining, rather than homologous recombination, generate these deletions. Satellite DNA sequences, one type of repetitive sequences, may mediate rearrangements by inducing DNA breakage followed by erroneous DNA repair (Page et al. 1996). Moreover, *Alu* repetitive elements have been shown to facilitate template switching during *Alu-Alu* mediated, disease associated rearrangements and in a yeast replicative repair assay (Boone et al. 2011; Boone et al. 2014; Mayle et al. 2015; Shaw and Lupski 2005). Sequencing of the breakpoints in LCR and repetitive sequences may provide further evidence consolidating the hypothesis that LCR facilitate replicative repair mediated rearrangements (Eid et al. 2009; English et al. 2015; Wang et al. 2015).

Unlike NAHR-mediated recurrent CNVs with boundaries confined by flanking LCRs, nonrecurrent CNVs may have variable boundaries and thus include variable gene content. An “atypical” CNV of a known genomic disorder may span additional genomic segments, change the copy number of neighboring dosage-sensitive disease-associated gene(s), and subsequently result in a complex phenotype blending multiple disorders (Table 3). For example, large, nonrecurrent duplications encompassing both *PMP22* and *RAI1* (*PMP22-RAI1* duplication) have been described in Yuan-Harel-Lupski syndrome (YUHAL, MIM: 616652) presenting a combination of Potocki-Lupski syndrome (PTLS, MIM# 610883) and Charcot-Marie-Tooth disease type 1A (CMT1A, MIM# 118220) traits with a potential more severe and early-onset peripheral neuropathy (Yuan et al. 2015a).

Here, we documented the reciprocal phenomenon: i.e. *PMP22-RAI1* deletions. A larger deletion may give rise to a complex phenotype by affecting multiple disease-associated loci via a combination of possibilities, including (1) spanning multiple loci associated with dominant disorders (Campbell et al. 2012), (2) unmasking recessive loci, and (3) causing gene fusion. For examples, large deletions spanning the tuberous sclerosis complex (TSC, MIM: 613254) gene *TSC2* (MIM: 191092) and autosomal dominant polycystic kidney disease (ADPKD, MIM: 173900) gene *PKD1* (MIM: 601313), two genes mapped “head-to-head” on chromosome 16p, result in early onset polycystic kidney disease with tuberous sclerosis (MIM: 600273), a mixed phenotype with both TSC and ADPKD traits (Brook-Carter et al. 1994). Similarly, large deletions resulting in more severe and complex phenotypes have been reported at other loci typically associated with recurrent deletions, such as the *BMPRIA* locus and the *STS* locus (Ballabio et al. 1989; van Bon et al. 2011). In rare circumstances of diffuse leiomyomatosis with Alport syndrome (MIM: 308940), a deletion includes part of *COL4A5* and results in Alport syndrome by a gene dosage-effect.

In addition, this deletion also spans part of the neighboring gene *COL4A6*, creates a fusion gene between *COL4A5* and *COL4A6* and thereby leads to diffuse leiomyomatosis (Table 3).

It is expected that subjects with a large deletion encompassing these two genes will manifest a resultant phenotype that consists of combined features of SMS and HNPP. Among our six subjects, two of them had a clear diagnosis of peripheral neuropathy at the time of latest examination. Some of these subjects were last examined during their first decade of life and thus may not have yet developed signs and symptoms of peripheral neuropathy. HNPP is an adult onset neuropathy that usually presents during the second or third decade. Early signs could have potentially been perceived on NCS; however, this test was not performed on every subject.

The severity of phenotype may correlate with the size of the deletion as well as the level of mosaicism. We observed a more severe neuropathy phenotype among the subjects with larger deletions (BABs 484, 608 and 993, and the subject reported by Goh *et al*), among which BAB993 had the smallest deletion of 6.9 Mb in size. These subjects were given a clinical diagnosis of HNPP, or otherwise had symptoms such as delayed walking, hyporeflexia, foot deformities and/or abnormal velocities on NCS. Even though there was a large age gap between some of these subjects, they showed signs of peripheral neuropathy during their first decade of life. Interestingly, subjects whose deletions were smaller than 6.9 Mb did not have NCS performed, possibly because these were not clinically indicated. Thus, even if their symptoms and clinical presentation of neuropathy seems much milder or even absent, we could not rule out motor and sensory abnormalities that could potentially be identified by NCS.

Mosaic deletions spanning both SMS and HNPP critical regions have been reported. These mosaic deletions were all uniquely observed, likely arose from postzygotic and mitotic rearrangements, and contributed to variable phenotypes. Quantified by fluorescence *in situ* hybridization, Goh *et al* reported a mosaic ~ 7.4 Mb *PMP22-RAI1* deletion with mosaicism level of 12% in a 15-year-9-month-old female subject with polyneuropathy and clinical diagnosis consistent with SMS (Fig. 3a). In our study, the mother of BAB484 (BAB485) harbored a mosaic *PMP22-RAI1* deletion with mosaicism level of 57% quantified by aCGH (Fig. 3b) (Zori et al. 1993). However, she had very mild phenotypes that were not consistent with SMS.

It is interesting that the level of mosaicism as measured in genomic DNA of cells isolated from blood did not seem to correlate with the severity of the phenotype in the two mosaic cases being compared. One possible explanation is that the organ distributions of mosaicism were different in these two individuals, with possible higher level in the neurological system of the subject reported by Goh *et al*. It needs to be considered that the patient reported by Goh *et al* also harbored a mosaic duplication on 17q. Although this duplication was not yet associated with a defined phenotype, it may aggravate the phenotype of this patient. These two observations, albeit limited data on only two subjects, might suggest that mosaic *PMP22-RAI1* deletion may have distinct phenotypic outcomes, ranging from nonsymptomatic to a phenotype with features combining both HNPP and SMS. Although mosaicism may not lead to clinical presentation of SMS and HNPP, it affects the recurrence

risk of the disorder in offspring. In a previous study, an unaffected mother with a mosaic deletion allele encompassing the last two exons of *RAI1* found in 25.1% of maternal blood cells, gave birth to three children with SMS that were affected half siblings, including a male patient with one father and fraternal male and female twins both with SMS from another father, with the same exonic deletion (Campbell et al. 2014). Therefore, molecular testing is recommended for the apparently unaffected parents with a child harboring nonrecurrent deletions to inform potential recurrence risk.

PMP22-RAI1 duplications outnumbered deletions. The *PMP22-RAI1* duplication was observed in 23/127 subjects with PTLs, in comparison to *PMP22-RAI1* deletion observed in 6/132 subjects with SMS (Fisher's Exact Test, $p < 0.001$). Brewer *et al* compared autosomal copy number duplication versus deletion generated from low-resolution chromosome analysis. Their studies revealed that the potential triplolethal regions (2.1% of the haploid autosomal length [HAL]) were less than the potential haplolethal regions (11% of HAL). Moreover, the longest duplication represented 4.3% of HAL in comparison to 2.7% of HAL for the longest deletion. Furthermore, the longer duplications/deletions are less frequently observed in the analyzed population (Brewer et al. 1998, 1999). These findings suggest that deletions are potentially less tolerated than duplications. Larger deletions, such as the *PMP22-RAI1* deletion, may be more lethal, because they have a higher chance to include more haploinsufficient genes or unmask recessive mutations on the other allele. The observance of one mosaic patient by Goh *et al.* (2014) also indicates possible cell lethality of the *PMP22-RAI1* deletion, which may include potential not-yet-identified essential genes mapping to this locus.

In summary, we documented six patients with nonrecurrent deletion encompassing both HNPP and SMS loci. Our genomic analyses suggest replicative mechanisms as a predominant mechanism underlying *PMP22-RAI1* contiguous gene deletion syndromes, i.e. both gains and losses, and provide further evidence supporting the role of complex genomic architecture in genomic instability. Detailed clinical profiling may contribute to the clinical management of this unique group of subjects. Furthermore, our study may contribute to the understanding of complex phenotypes involving multiple disease-associated loci from a mechanistic standpoint.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Bai Y, Zhang X, Katona I, Saporta MA, Shy ME, O'Malley HA, Isom LL, Suter U, Li J. Conduction block in PMP22 deficiency. *J Neurosci*. 2010; 30:600–8. DOI: 10.1523/JNEUROSCI.4264-09.2010 [PubMed: 20071523]
- Ballabio A, Bardoni B, Carrozzo R, Andria G, Bick D, Campbell L, Hamel B, Ferguson-Smith MA, Gimelli G, Fraccaro M, et al. Contiguous gene syndromes due to deletions in the distal short arm of the human X chromosome. *Proc Natl Acad Sci U S A*. 1989; 86:10001–5. [PubMed: 2602357]
- Bi W, Saifi GM, Girirajan S, Shi X, Szomju B, Firth H, Magenis RE, Potocki L, Elsea SH, Lupski JR. RAI1 point mutations, CAG repeat variation, and SNP analysis in non-deletion Smith-Magenis syndrome. *Am J Med Genet A*. 2006; 140:2454–63. DOI: 10.1002/ajmg.a.31510 [PubMed: 17041942]
- Bi W, Yan J, Stankiewicz P, Park SS, Walz K, Boerkoel CF, Potocki L, Shaffer LG, Devriendt K, Nowaczyk MJ, Inoue K, Lupski JR. Genes in a refined Smith-Magenis syndrome critical deletion interval on chromosome 17p11.2 and the syntenic region of the mouse. *Genome Res*. 2002; 12:713–28. DOI: 10.1101/gr.73702 [PubMed: 11997338]
- Boone PM, Liu P, Zhang F, Carvalho CM, Towne CF, Batish SD, Lupski JR. Alu-specific microhomology-mediated deletion of the final exon of SPAST in three unrelated subjects with hereditary spastic paraplegia. *Genet Med*. 2011; 13:582–92. DOI: 10.1097/GIM.0b013e3182106775 [PubMed: 21659953]
- Boone PM, Yuan B, Campbell IM, Scull JC, Withers MA, Baggett BC, Beck CR, Shaw CJ, Stankiewicz P, Moretti P, Goodwin WE, Hein N, Fink JK, Seong MW, Seo SH, Park SS, Karbassi ID, Batish SD, Ordonez-Ugalde A, Quintans B, Sobrido MJ, Stemmler S, Lupski JR. The Alu-rich genomic architecture of SPAST predisposes to diverse and functionally distinct disease-associated CNV alleles. *Am J Hum Genet*. 2014; 95:143–61. DOI: 10.1016/j.ajhg.2014.06.014 [PubMed: 25065914]
- Brewer C, Holloway S, Zawalnyski P, Schinzel A, FitzPatrick D. A chromosomal deletion map of human malformations. *Am J Hum Genet*. 1998; 63:1153–9. DOI: 10.1086/302041 [PubMed: 9758599]
- Brewer C, Holloway S, Zawalnyski P, Schinzel A, FitzPatrick D. A chromosomal duplication map of malformations: regions of suspected haplo- and triplolethality- and tolerance of segmental aneuploidy--in humans. *Am J Hum Genet*. 1999; 64:1702–8. DOI: 10.1086/302410 [PubMed: 10330358]
- Brook-Carter PT, Peral B, Ward CJ, Thompson P, Hughes J, Maheshwar MM, Nellist M, Gamble V, Harris PC, Sampson JR. Deletion of the TSC2 and PKD1 genes associated with severe infantile polycystic kidney disease--a contiguous gene syndrome. *Nat Genet*. 1994; 8:328–32. DOI: 10.1038/ng1294-328 [PubMed: 7894481]
- Campbell IM, Yatsenko SA, Hixson P, Reimschisel T, Thomas M, Wilson W, Dayal U, Wheless JW, Crunk A, Curry C, Parkinson N, Fishman L, Riviello JJ, Nowaczyk MJ, Zeesman S, Rosenfeld JA, Bejjani BA, Shaffer LG, Cheung SW, Lupski JR, Stankiewicz P, Scaglia F. Novel 9q34.11 gene deletions encompassing combinations of four Mendelian disease genes: STXBP1, SPTAN1, ENG, and TOR1A. *Genet Med*. 2012; 14:868–76. DOI: 10.1038/gim.2012.65 [PubMed: 22722545]
- Campbell IM, Yuan B, Robberecht C, Pfundt R, Szafranski P, McEntagart ME, Nagamani SC, Erez A, Bartnik M, Wisniewiecka-Kowalik B, Plunkett KS, Pursley AN, Kang SH, Bi W, Lalani SR, Bacino CA, Vast M, Marks K, Patton M, Olofsson P, Patel A, Veltman JA, Cheung SW, Shaw CA, Vissers LE, Vermeesch JR, Lupski JR, Stankiewicz P. Parental somatic mosaicism is underrecognized and influences recurrence risk of genomic disorders. *Am J Hum Genet*. 2014; 95:173–82. DOI: 10.1016/j.ajhg.2014.07.003 [PubMed: 25087610]
- Carvalho CM, Lupski JR. Mechanisms underlying structural variant formation in genomic disorders. *Nat Rev Genet*. 2016; 17:224–38. DOI: 10.1038/nrg.2015.25 [PubMed: 26924765]
- Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, Swanson PD, Odelberg SJ, Distèche CM, Bird TD. DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell*. 1993; 72:143–51. [PubMed: 8422677]

- Chen KS, Manian P, Koeuth T, Potocki L, Zhao Q, Chinault AC, Lee CC, Lupski JR. Homologous recombination of a flanking repeat gene cluster is a mechanism for a common contiguous gene deletion syndrome. *Nat Genet.* 1997; 17:154–63. DOI: 10.1038/ng1097-154 [PubMed: 9326934]
- Chrestian N, McMillan H, Poulin C, Campbell C, Vajsar J. Hereditary neuropathy with liability to pressure palsies in childhood: Case series and literature update. *Neuromuscul Disord.* 2015; 25:693–8. DOI: 10.1016/j.nmd.2015.06.463 [PubMed: 26189194]
- De Jong JGY. Over families met hereditaire dispositie tot het optreden van neuritiden, gecorreleerd met migraine. *Psychiat Neurol Bl.* 1947; 50:60–76.
- Dittwald P, Gambin T, Szafranski P, Li J, Amato S, Divon MY, Rodriguez Rojas LX, Elton LE, Scott DA, Schaaf CP, Torres-Martinez W, Stevens AK, Rosenfeld JA, Agadi S, Francis D, Kang SH, Breman A, Lalani SR, Bacino CA, Bi W, Milosavljevic A, Beaudet AL, Patel A, Shaw CA, Lupski JR, Gambin A, Cheung SW, Stankiewicz P. NAHR-mediated copy-number variants in a clinical population: mechanistic insights into both genomic disorders and Mendelizing traits. *Genome Res.* 2013; 23:1395–409. DOI: 10.1101/gr.152454.112 [PubMed: 23657883]
- Edelman EA, Girirajan S, Finucane B, Patel PI, Lupski JR, Smith AC, Elsea SH. Gender, genotype, and phenotype differences in Smith-Magenis syndrome: a meta-analysis of 105 cases. *Clin Genet.* 2007; 71:540–50. DOI: 10.1111/j.1399-0004.2007.00815.x [PubMed: 17539903]
- Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Korlach J, Turner S. Real-time DNA sequencing from single polymerase molecules. *Science.* 2009; 323:133–8. DOI: 10.1126/science.1162986 [PubMed: 19023044]
- English AC, Salerno WJ, Hampton OA, Gonzaga-Jauregui C, Ambreth S, Ritter DI, Beck CR, Davis CF, Dahdouli M, Ma S, Carroll A, Veeraraghavan N, Bruestle J, Drees B, Hastie A, Lam ET, White S, Mishra P, Wang M, Han Y, Zhang F, Stankiewicz P, Wheeler DA, Reid JG, Muzny DM, Rogers J, Sabo A, Worley KC, Lupski JR, Boerwinkle E, Gibbs RA. Assessing structural variation in a personal genome-towards a human reference diploid genome. *BMC Genomics.* 2015; 16:286.doi: 10.1186/s12864-015-1479-3 [PubMed: 25886820]
- Gabreels-Festen AA, Gabreels FJ, Joosten EM, Vingerhoets HM, Renier WO. Hereditary neuropathy with liability to pressure palsies in childhood. *Neuropediatrics.* 1992; 23:138–43. [PubMed: 1322507]
- Girirajan S, Vlangos CN, Szomju BB, Edelman E, Trevors CD, Dupuis L, Nezarati M, Bunyan DJ, Elsea SH. Genotype-phenotype correlation in Smith-Magenis syndrome: evidence that multiple genes in 17p11.2 contribute to the clinical spectrum. *Genet Med.* 2006; 8:417–27. DOI: 10.109701/gim.0000228215.32110.89 [PubMed: 16845274]
- Goh ES, Banwell B, Stavropoulos DJ, Shago M, Yoon G. Mosaic microdeletion of 17p11.2-p12 and duplication of 17q22-q24 in a girl with Smith-Magenis phenotype and peripheral neuropathy. *Am J Med Genet A.* 2014; 164A:748–52. DOI: 10.1002/ajmg.a.36322 [PubMed: 24357149]
- Greenberg F, Guzzetta V, Montes de Oca-Luna R, Magenis RE, Smith AC, Richter SF, Kondo I, Dobyns WB, Patel PI, Lupski JR. Molecular analysis of the Smith-Magenis syndrome: a possible contiguous-gene syndrome associated with del(17)(p11.2). *Am J Hum Genet.* 1991; 49:1207–18. [PubMed: 1746552]
- Greenberg F, Lewis RA, Potocki L, Glaze D, Parke J, Killian J, Murphy MA, Williamson D, Brown F, Dutton R, McCluggage C, Friedman E, Sulek M, Lupski JR. Multi-disciplinary clinical study of Smith-Magenis syndrome (deletion 17p11.2). *Am J Med Genet.* 1996; 62:247–54. DOI: 10.1002/(SICI)1096-8628(19960329)62:3<247::AID-AJMG9>3.0.CO;2-Q [PubMed: 8882782]
- Hastings PJ, Ira G, Lupski JR. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet.* 2009; 5:e1000327.doi: 10.1371/journal.pgen.1000327 [PubMed: 19180184]
- Juyal RC, Figueroa LE, Hauge X, Elsea SH, Lupski JR, Greenberg F, Baldini A, Patel PI. Molecular analyses of 17p11.2 deletions in 62 Smith-Magenis syndrome patients. *Am J Hum Genet.* 1996; 58:998–1007. [PubMed: 8651284]

- Lacaria M, Saha P, Potocki L, Bi W, Yan J, Girirajan S, Burns B, Elsea S, Walz K, Chan L, Lupski JR, Gu W. A duplication CNV that conveys traits reciprocal to metabolic syndrome and protects against diet-induced obesity in mice and men. *PLoS Genet.* 2012; 8:e1002713.doi: 10.1371/journal.pgen.1002713 [PubMed: 22654670]
- Lee JA, Carvalho CM, Lupski JR. A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell.* 2007; 131:1235–47. DOI: 10.1016/j.cell.2007.11.037 [PubMed: 18160035]
- Li J, Ghandour K, Radovanovic D, Shy RR, Krajewski KM, Shy ME, Nicholson GA. Stoichiometric alteration of PMP22 protein determines the phenotype of hereditary neuropathy with liability to pressure palsies. *Arch Neurol.* 2007; 64:974–8. DOI: 10.1001/archneur.64.7.974 [PubMed: 17620487]
- Liburd N, Ghosh M, Riazuddin S, Naz S, Khan S, Ahmed Z, Riazuddin S, Liang Y, Menon PS, Smith T, Smith AC, Chen KS, Lupski JR, Wilcox ER, Potocki L, Friedman TB. Novel mutations of MYO15A associated with profound deafness in consanguineous families and moderately severe hearing loss in a patient with Smith-Magenis syndrome. *Hum Genet.* 2001; 109:535–41. DOI: 10.1007/s004390100604 [PubMed: 11735029]
- Liu P, Lacaria M, Zhang F, Withers M, Hastings PJ, Lupski JR. Frequency of nonallelic homologous recombination is correlated with length of homology: evidence that ectopic synapsis precedes ectopic crossing-over. *Am J Hum Genet.* 2011; 89:580–8. DOI: 10.1016/j.ajhg.2011.09.009 [PubMed: 21981782]
- Lupianez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R, Santos-Simarro F, Gilbert-Dussardier B, Wittler L, Borschiwer M, Haas SA, Osterwalder M, Franke M, Timmermann B, Hecht J, Spielmann M, Visel A, Mundlos S. Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. *Cell.* 2015; 161:1012–25. DOI: 10.1016/j.cell.2015.04.004 [PubMed: 25959774]
- Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet.* 1998; 14:417–22. [PubMed: 9820031]
- Lupski JR. Genomic disorders ten years on. *Genome Med.* 2009; 1:42.doi: 10.1186/gm42 [PubMed: 19439022]
- Lupski JR. Structural variation mutagenesis of the human genome: Impact on disease and evolution. *Environ Mol Mutagen.* 2015; 56:419–436. DOI: 10.1002/em.21943 [PubMed: 25892534]
- Lupski JR, Stankiewicz P. Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. *PLoS Genet.* 2005; 1:e49.doi: 10.1371/journal.pgen.0010049 [PubMed: 16444292]
- Mayle R, Campbell IM, Beck CR, Yu Y, Wilson M, Shaw CA, Bjergbaek L, Lupski JR, Ira G. DNA REPAIR. Mus81 and converging forks limit the mutagenicity of replication fork breakage. *Science.* 2015; 349:742–7. DOI: 10.1126/science.aaa8391 [PubMed: 26273056]
- McVey M, Lee SE. MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. *Trends Genet.* 2008; 24:529–38. DOI: 10.1016/j.tig.2008.08.007 [PubMed: 18809224]
- Neira-Fresneda J, Potocki L. Neurodevelopmental Disorders Associated with Abnormal Gene Dosage: Smith–Magenis and Potocki–Lupski Syndromes. *Journal of Pediatric Genetics.* 2015; 04:159–167. DOI: 10.1055/s-0035-1564443 [PubMed: 27617127]
- Page SL, Shin JC, Han JY, Choo KH, Shaffer LG. Breakpoint diversity illustrates distinct mechanisms for Robertsonian translocation formation. *Hum Mol Genet.* 1996; 5:1279–88. [PubMed: 8872467]
- Pentao L, Wise CA, Chinault AC, Patel PI, Lupski JR. Charcot-Marie-Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. *Nat Genet.* 1992; 2:292–300. DOI: 10.1038/ng1292-292 [PubMed: 1303282]
- Potocki L, Chen KS, Koeuth T, Killian J, Iannaccone ST, Shapira SK, Kashork CD, Spikes AS, Shaffer LG, Lupski JR. DNA rearrangements on both homologues of chromosome 17 in a mildly delayed individual with a family history of autosomal dominant carpal tunnel syndrome. *Am J Hum Genet.* 1999; 64:471–8. DOI: 10.1086/302240 [PubMed: 9973284]
- Ricard G, Molina J, Chrast J, Gu W, Gheldof N, Pradervand S, Schutz F, Young JI, Lupski JR, Reymond A, Walz K. Phenotypic consequences of copy number variation: insights from Smith-

- Magenis and Potocki-Lupski syndrome mouse models. *PLoS Biol.* 2010; 8:e1000543.doi: 10.1371/journal.pbio.1000543
- Shaw CJ, Lupski JR. Non-recurrent 17p11.2 deletions are generated by homologous and non-homologous mechanisms. *Hum Genet.* 2005; 116:1–7. DOI: 10.1007/s00439-004-1204-9 [PubMed: 15526218]
- Slager RE, Newton TL, Vlangos CN, Finucane B, Elsea SH. Mutations in *RAI1* associated with Smith-Magenis syndrome. *Nat Genet.* 2003; 33:466–8. DOI: 10.1038/ng1126 [PubMed: 12652298]
- Staal A, de Weerd CJ, Went LN. Hereditary compression syndrome of peripheral nerves. *Neurology.* 1965; 15:1008–17. [PubMed: 4285163]
- Stankiewicz P, Lupski JR. Structural variation in the human genome and its role in disease. *Annu Rev Med.* 2010; 61:437–55. DOI: 10.1146/annurev-med-100708-204735 [PubMed: 20059347]
- Stankiewicz P, Shaw CJ, Dapper JD, Wakui K, Shaffer LG, Withers M, Elizondo L, Park SS, Lupski JR. Genome architecture catalyzes nonrecurrent chromosomal rearrangements. *Am J Hum Genet.* 2003; 72:1101–16. DOI: 10.1086/374385 [PubMed: 12649807]
- Szafranski P, Gambin T, Dharmadhikari AV, Akdemir KC, Jhangiani SN, Schuette J, Godiwala N, Yatsenko SA, Sebastian J, Madan-Khetarpal S, Surti U, Abellar RG, Bateman DA, Wilson AL, Markham MH, Slamon J, Santos-Simarro F, Palomares M, Nevado J, Lapunzina P, Chung BH, Wong WL, Chu YW, Mok GT, Kerem E, Reiter J, Ambalavanan N, Anderson SA, Kelly DR, Shieh J, Rosenthal TC, Scheible K, Steiner L, Iqbal MA, McKinnon ML, Hamilton SJ, Schlade-Bartusiak K, English D, Henderson G, Roeder ER, DeNapoli TS, Littlejohn RO, Wolff DJ, Wagner CL, Yeung A, Francis D, Fiorino EK, Edelman M, Fox J, Hayes DA, Janssens S, De Baere E, Menten B, Loccufier A, Vanwalleghem L, Moerman P, Sznajder Y, Lay AS, Kussmann JL, Chawla J, Payton DJ, Phillips GE, Brosens E, Tibboel D, de Klein A, Maystadt I, Fisher R, Sebire N, Male A, Chopra M, Pinner J, Malcolm G, Peters G, Arbuckle S, Lees M, Mead Z, Quarrell O, Sayers R, Owens M, Shaw-Smith C, Lioy J, McKay E, de Leeuw N, Feenstra I, Spruijt L, Elmslie F, Thiruchelvam T, Bacino CA, Langston C, Lupski JR, Sen P, Popek E, Stankiewicz P. Pathogenetics of alveolar capillary dysplasia with misalignment of pulmonary veins. *Hum Genet.* 2016; doi: 10.1007/s00439-016-1655-9
- Trask BJ, Mefford H, van den Engh G, Massa HF, Juyal RC, Potocki L, Finucane B, Abuelo DN, Witt DR, Magenis E, Baldini A, Greenberg F, Lupski JR, Patel PI. Quantification by flow cytometry of chromosome-17 deletions in Smith-Magenis syndrome patients. *Hum Genet.* 1996; 98:710–8. [PubMed: 8931707]
- van Bon BW, Balciuniene J, Fruhman G, Nagamani SC, Broome DL, Cameron E, Martinet D, Roulet E, Jacquemont S, Beckmann JS, Irons M, Potocki L, Lee B, Cheung SW, Patel A, Bellini M, Selicorni A, Ciccone R, Silengo M, Vetro A, Knoers NV, de Leeuw N, Pfundt R, Wolf B, Jira P, Aradhya S, Stankiewicz P, Brunner HG, Zuffardi O, Selleck SB, Lupski JR, de Vries BB. The phenotype of recurrent 10q22q23 deletions and duplications. *Eur J Hum Genet.* 2011; 19:400–8. DOI: 10.1038/ejhg.2010.211 [PubMed: 21248748]
- Wang M, Beck CR, English AC, Meng Q, Buhay C, Han Y, Doddapaneni HV, Yu F, Boerwinkle E, Lupski JR, Muzny DM, Gibbs RA. PacBio-LITS: a large-insert targeted sequencing method for characterization of human disease-associated chromosomal structural variations. *BMC Genomics.* 2015; 16:214.doi: 10.1186/s12864-015-1370-2 [PubMed: 25887218]
- Wu N, Ming X, Xiao J, Wu Z, Chen X, Shinawi M, Shen Y, Yu G, Liu J, Xie H, Gucev ZS, Liu S, Yang N, Al-Kateb H, Chen J, Zhang J, Hauser N, Zhang T, Tasic V, Liu P, Su X, Pan X, Liu C, Wang L, Shen J, Shen J, Chen Y, Zhang T, Zhang J, Choy KW, Wang J, Wang Q, Li S, Zhou W, Guo J, Wang Y, Zhang C, Zhao H, An Y, Zhao Y, Wang J, Liu Z, Zuo Y, Tian Y, Weng X, Sutton VR, Wang H, Ming Y, Kulkarni S, Zhong TP, Giampietro PF, Dunwoodie SL, Cheung SW, Zhang X, Jin L, Lupski JR, Qiu G, Zhang F. TBX6 null variants and a common hypomorphic allele in congenital scoliosis. *N Engl J Med.* 2015; 372:341–50. DOI: 10.1056/NEJMoa1406829 [PubMed: 25564734]
- Yan J, Bi W, Lupski JR. Penetrance of craniofacial anomalies in mouse models of Smith-Magenis syndrome is modified by genomic sequence surrounding *Rai1*: not all null alleles are alike. *Am J Hum Genet.* 2007; 80:518–25. DOI: 10.1086/512043 [PubMed: 17273973]

- Yuan B, Harel T, Gu S, Liu P, Burglen L, Chantot-Bastaraud S, Gelowani V, Beck CR, Carvalho CM, Cheung SW, Coe A, Malan V, Munnich A, Magoulas PL, Potocki L, Lupski JR. Nonrecurrent 17p11.2p12 Rearrangement Events that Result in Two Concomitant Genomic Disorders: The PMP22-RAI1 Contiguous Gene Duplication Syndrome. *Am J Hum Genet.* 2015a; 97:691–707. DOI: 10.1016/j.ajhg.2015.10.003 [PubMed: 26544804]
- Yuan B, Liu P, Gupta A, Beck CR, Tejomurtula A, Campbell IM, Gambin T, Simmons AD, Withers MA, Harris RA, Rogers J, Schwartz DC, Lupski JR. Comparative Genomic Analyses of the Human NPHP1 Locus Reveal Complex Genomic Architecture and Its Regional Evolution in Primates. *PLoS Genet.* 2015b; 11:e1005686.doi: 10.1371/journal.pgen.1005686 [PubMed: 26641089]
- Zhang F, Khajavi M, Connolly AM, Towne CF, Batish SD, Lupski JR. The DNA replication FoSTeS/MMBIR mechanism can generate genomic, genic and exonic complex rearrangements in humans. *Nat Genet.* 2009; 41:849–53. DOI: 10.1038/ng.399 [PubMed: 19543269]
- Zori RT, Lupski JR, Heju Z, Greenberg F, Killian JM, Gray BA, Driscoll DJ, Patel PI, Zackowski JL. Clinical, cytogenetic, and molecular evidence for an infant with Smith-Magenis syndrome born from a mother having a mosaic 17p11.2p12 deletion. *Am J Med Genet.* 1993; 47:504–11. DOI: 10.1002/ajmg.1320470414 [PubMed: 8256814]

Fig. 1. Summary diagram of the genomic rearrangements identified in 17p in the cohort of 132 subjects with SMS

A. Ideogram showing the entire chromosome 17. The region included in the summary diagram is indicated by a red box. **B.** The genomic rearrangements are shown. The plots are arranged according to the starting coordinate of the rearrangements identified in each case. Subjects are identified with common recurrent SMS deletion (N=96), uncommon recurrent deletion 1 (N=8), uncommon recurrent deletion 2 (N=2), uncommon recurrent deletion 3 (N=2) and nonrecurrent deletion (N=24). The annotations in the figure are the same as Fig. 2. The BAB numbers in red harbor *PMP22-RAI1* deletions. The incidence counts of each genomic rearrangement are shown on the right panel. The incidence is one for the rearrangements labeled with BAB numbers. Segments in green, heterozygous deletion; dark green, region of uncertainty; white, copy number neutral regions. Colored vertical lines represent genomic location of critical genes or LCR clusters. Vertical lines in light blue, CMT1A-REPs; magenta, SMS-REPs; dark blue, *RNU3* LCRs; maroon, 17p-PROX; grey, *PMP22* and *RAI1*. CR, common recurrent SMS deletion; UR1, UR2 and UR3 stands for uncommon recurrent deletion 1, 2 and 3 respectively.

Fig. 2. Array CGH plot of *PMP22-RAI1* deletion identified in six subjects

A. The plots are arranged according to the starting coordinate of the deletion identified in each case. Red ($\log_2$ ratio > 0.25), black ($-0.25 < \log_2$ ratio < 0.25) and green ($\log_2$ ratio < -0.25) dots denote array CGH probes. Grey vertical lines mark position of genes *PMP22* and *RAI1*; green vertical lines mark the distal and proximal boundary of SRO. **B.** Breakpoint junction sequences of BAB2011 and BAB8499. Sequences in red are matching sequences at the proximal (PROX) and distal (DIST) end. Sequences in purple represent microhomologies. The genomic coordinates of the breakpoint junction are denoted.

Fig. 3. Constitutional and mosaic *PMP22-RAI1* deletions

A. Summary diagram comparing the deletion in six cases of our study with a reported case with mosaic deletion. The mosaic deletion is with light green color. The asterisks (*) mark mosaic cases. **B.** Transmission of a *PMP22-RAI1* deletion from mother (BAB485) to daughter (BAB484). The green horizontal line demarcates the aCGH log₂ ratio of the deletion in each subject.

Table 1

Characteristics of the *PMP22-RAI1* deletions.

BAB	Inheritance	Breakpoint junction (JCT)			Ref
		Coordinates (Chr17)	Size (Mb)	Features	
484	Maternal	13539851-21506107 (Min) 13537876-21701186 (Max)	8.0	Proximal boundary is in 17p-PROX.	Zori <i>et al</i> (1993)
608	Not Maternal	12110198-21506107 (Min) 12109786-21701186 (Max)	9.4	Proximal boundary is in 17p-PROX.	Juyal <i>et al</i> (1996)
993	<i>de novo</i>	14564592-21506107 (Min) 14555246-21701186 (Max)	6.9	Proximal boundary is in 7p-PROX.	Stankiewicz <i>et al</i> (2003)
2011	<i>de novo</i>	15116741-19716192 (exact)	4.6	1 base pair (C) microhomology at the JCT.	Liu <i>et al</i> (2011)
8499	Not maternal	12068399-17911184 (exact)	5.8	6 base pair (ACCACC) microhomology at the JCT.	-
8501	<i>de novo</i>	13845359-18928347 (Min) 13845043-19140987 (Max)	5.1	Proximal boundary is in an LCR cluster.	-

Table 2

Clinical Information of the Subjects with *PMP22-RAI1* deletions.

Identifier (BAB numbers) Reference	Germline				Mosaic			
	484	608	993	2011	8499 (SMS530)	8501	485	Goh <i>et al</i>
	Zori <i>et al</i> (1993) **	Juyal <i>et al</i> (1996) *	Stankiewicz <i>et al</i> (2003) *	Liu <i>et al</i> (2011) *	-	-	Zori <i>et al</i> (1993) **	Goh <i>et al</i> (2014) **
Sex	Female	Female	Female	Male	Male	Female	Female	Female
Age at last exam	6 y	22 y	3 y	9 y	24 y	23 y	22 y	15 y
Deletion size (Mb)	8.0	9.4	6.9	4.6	5.8	5.1	80.0 (mosaic, 57%)	7.4 (mosaic, 12%)
Infancy	+	-	-	NR	+	-	+	NR
Development								
Feeding difficulties /FTT	+	-	+	+	+	+	-	+
Infantile hypotonia	+	-	+	+	+	+	-	+
Intellectual disability/IQ	+ /NR	+ /NR	NR	+ /48	+ /57	+ /NR	+ /88	+
Motor delay/Age at walking	+ /28 mos	+ /36 mos	+ /NR	+ /2 years	+ /19 mos	+ /2 years	-	+ /18 mos
Speech delay	+	+	+	+	+	+	-	+
Behavioral difficulties	+	+	+	+	+	+	-	-
Facial dysmorphism	+	+	+	+	+	+	+	+
Short stature	+	+	+	+	+	+	-	-
Scoliosis	+	+	+	-	+	+	+	-
Brachydactyly	+	+	+	+	+	NR	-	+
Syndactyly	+	+	-	-	+	+	+	+
Sensory loss	NR	NR	-	-	+	NR	-	+
DTRs (+)	1+	2+	2+	2+	NR	2+	2+	Absent
Foot deformities	Out-Toeing R foot, small ankles, "Stork legs"	-	Pes Planus	-	Pes Planus	-	Pes Cavus	Pes Cavus
Unusual gait	NR	-	+	+	+	-	NR	+
Orthotics/Therapy	+	R tendon Achilles lengthening	NR	+	ShI	+	NR	NR
Other congenital anomalies								
Ocular Anomalies	ST	IrA	MYOP, IrA	NR	ST, MYOP, RD	ST, ASTIGM	IrA	MYOP
Congenital heart defect	ASD	ASD, VSD, PS	-	NR	-	-	-	-

Studies and imaging	Renal abnormality	Brain MRI abnormality	Germline		Mosaic	
NCS			NR	-	NR	-
			NR	Minimal prominence of the ventricular system.	NR	Prominent ventricles and asymmetry of the calvaria.
	Age at the time of study	28mos/47mos	18 y	3 y	NR	7.5 y
	Median nerve motor MCV (m/sec) (>39)	23/60	NR	45	NR	NR
	Median Distal Latency (msec)	3.4/2	NR	2.7	NR	4
	Median SCV (m/sec)	47.5 /NR	NR	50	NR	NR
	Peroneal Nerve motor MCV (m/sec) (>39)	43/51	36	50	NR	58
	Peroneal nerve distal latency	5/2.8	6	2	NR	5.6

* Only molecular data was reported.

*** molecular and clinical information were reported.

ASD: atrial septal defect, ASTIG: astigmatism, DTRs: deep tendon reflexes, FTT: failure to thrive, IrA: iris abnormalities, IQ: intelligence quotient, MCV: motor conduction velocity, mos: months, MRI: magnetic resonance imaging, MYOP: myopia, NCS: nerve conduction studies, NR: no record, PS: pulmonic stenosis, RD: retinal detachment, SCV: sensory conduction velocity, ShI: shoe inserts, ST: strabismus VSD: ventricular septal defect. Details are included in Table S1.

Examples of contiguous gene duplication/deletion involving multiple disease-associated loci.

Phenotypes	Mechanism	Components	Genes	Mechanism	Inheritance
Yuan-Harel-Lupski syndrome (MIM: 616652); a complex neurodevelopmental disorder characterized by global developmental delay and early-onset peripheral neuropathy	Contiguous gene duplication	Charcot-Marie-Tooth disease type 1A (MIM: 118220)	<i>PMP22</i>	NAHR-mediated recurrent duplication	AD
Polycystic kidney disease, infantile severe, with tuberous sclerosis (MIM: 600273)	Contiguous gene deletion	Potocki-Lupski syndrome (MIM: 610883) Polycystic kidney disease, adult type 1 (MIM: 173900)	<i>RAI1</i> <i>PKD1</i>	NAHR-mediated recurrent duplication Point/truncating mutation	AD AD
Chromosome 10q22.3-q23.2 deletion syndrome (MIM: 612242); a more severe phenotype with infantile/juvenile polyposis, macrocephaly, dysmorphic facial features, and developmental delay.	Contiguous gene deletion	Tuberous sclerosis-2 (MIM: 613254) dysmorphic facies, developmental delay, and multiple congenital anomalies	<i>TSC2</i> <i>BMP1A</i>	Point/truncating mutation NAHR-mediated recurrent deletion	AD AD
X-linked ichthyosis, intellectual disability, Kallmann syndrome (comprising hypogonadotropic hypogonadism and anosmia), features of X-linked chondrodysplasia punctata, short stature, and/or ocular albinism.	Contiguous gene deletion, X-linked recessive	Cowden syndrome 1 (MIM: 158350) X-linked ichthyosis (MIM: 308100)	<i>PTEN</i> <i>STS</i>	Point/truncating mutation NAHR-mediated recurrent deletion	AD XLR
Diffuse leiomyomatosis with Alport syndrome (MIM: 308940)	Fusion gene	Kallmann syndrome (MIM: 308700) X-linked chondrodysplasia punctata (MIM: 302950) X-linked idiopathic short stature (MIM: 312865) Ocular albinism (MIM: 300500) Alport syndrome (MIM: 301050)	<i>KAL1</i> <i>ARSE</i> <i>SHOX</i> <i>GPR143</i> <i>COL4A5</i>	Point/truncating mutation Point/truncating mutation Point/truncating mutation Point/truncating mutation Point/truncating mutation	XL XLR XL XL XLD
		Deafness, X-linked 6 (MIM: 300914)	<i>COL4A6</i>	Point mutation	XLR

AD: autosomal dominant; XL, X-linked; XLR, X-linked recessive.