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FIGURE LEGENDS

FIGURE 1. Facial appearance of patients with *RAF1* mutations. a-f: patients with p.S257L mutations. a, NS135; b, NS146; c, NS215; d, NS256; NS258 at 6 months (e) and 2 years and 4 months (f); g, NS222 with p.S427G.

FIGURE 2. Analysis of phosphorylation status, degradation and effect on downstream signaling in RAF1 mutants identified in this study.
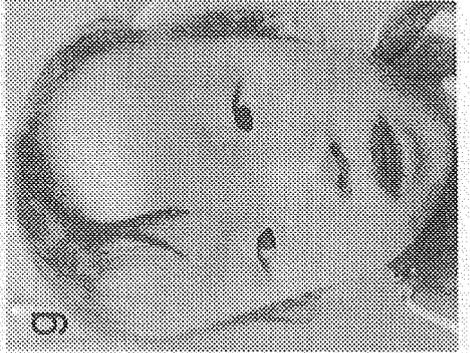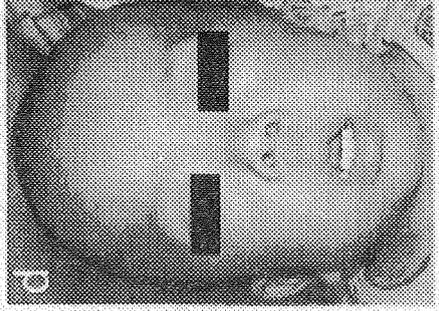
A: Phosphorylation status of wild-type (WT) RAF1 and mutants. Expression levels of RAF1 proteins and their phosphorylation levels were detected with different antibodies indicated in the figure. Transfection efficiency was measured using anti-neomycin phosphotransferase II (α-Neo) antibody. The arrow indicates the serine-phosphorylated expressed RAF1. B: Phosphorylation of S259 was confirmed by immunoprecipitation. Myc-tagged RAF1 was immunoprecipitated using anti-Myc antibody and the phosphorylation of S259 was determined. C: Time course experiments of WT RAF1 and p.S427G. The RAF1 protein was detected using anti-Myc antibody (clone 4A6, Millipore). FBS, fetal bovine serum. D: ELK transactivation in WT and mutants. Results are expressed as the means and standard deviations of mean values from triplicate samples. A significant increase in relative luciferase activity (RLA) was observed in cells transfected with p.S257L, p. N262K and p.S427G, but not in cells transfected with p.H103Q or p.R191I. WT, wild-type; *$P<0.01$ by Student's *t*-test.

FIGURE 3. Phosphorylation of S259, binding to 14-3-3 and ERK activation of mutants located in the CR2 domain. A: Phosphorylation status of WT and mutants located in the CR2 domain.
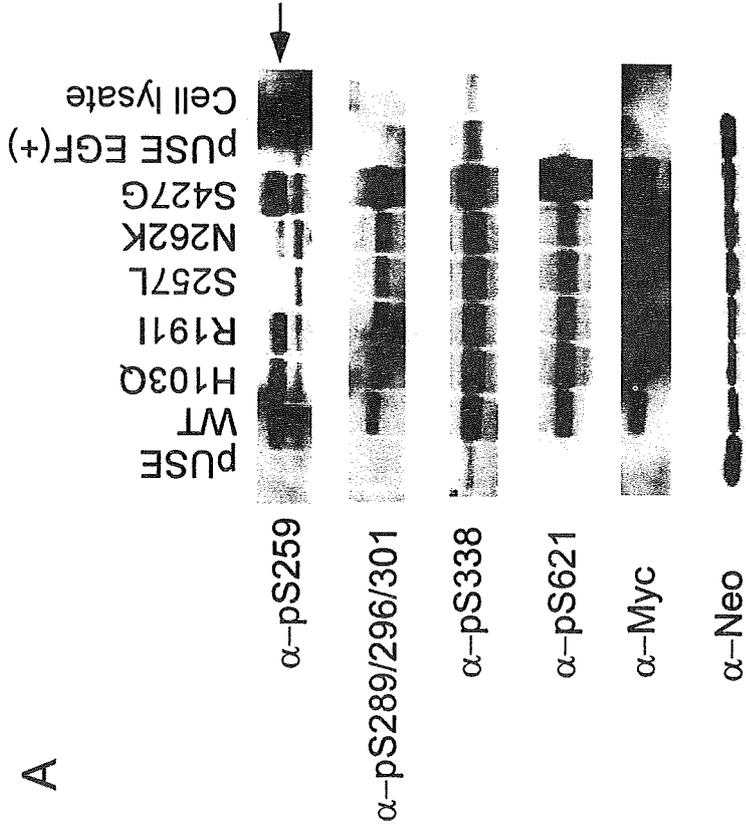
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Phosphorylation of S259 was not observed in cells expressing p.S257L, p.S259F, p.P261A and

p.N262K. In order to examine the level of full activation of ERK, mock-transfected cells were

treated with 10 ng/ml EGF. ERK activation was observed in cells expressing p.S257L, p.S259F,

p.P261A and p.N262K, but was weaker than those in cells expressing p.S427G and EGF-treated

cells. The arrow indicates the serine-phosphorylated expressed RAF1. B: Epitope mapping of the

anti-pRAF1 (S259) antibody using a solid-phase immunoassay. The antibody was able to

recognize peptides with S257L or N262K mutations when S259 was phosphorylated, but was not

able to recognize peptides without Ser259 phosphorylation. Results are expressed as the means

and standard deviations of mean values from triplicate samples. C: Binding of RAF-1 to

14-3-3ζ. HEK293 cells were transfected with constructs harboring FLAG-tagged 14-3-3 and

one construct of pUSE WT, p.S257L/ p.S621A or p.N262K/ p.S621A. Immunoprecipitation was

performed using anti-Myc antibody, and 14-3-3 binding was determined by anti-FLAG antibody

(upper panel). Phosphorylation of S259 and RAF1 expression were determined in cell lysates

used for the immunoprecipitation (lower panel). The arrow indicates the band for 14-3-3. D:

Binding of 14-3-3ζ to RAF-1. Immunoprecipitation was performed using anti-FLAG antibody

and RAF1 binding was examined using anti-RAF1 antibody (Upper panel). The binding of

14-3-3 to endogenous RAF1 was scarcely observed (lane 1, pUSE). Phosphorylation of S259 and

RAF1 expression were determined using cell lysates used for the immunoprecipitation (lower

panel). E: Domain organization and the distribution of mutations in RAF1 protein. The three

regions conserved in all RAF proteins (conserved region (CR) 1, CR2, and CR3) are shown in

pink. Mutations identified in this study are shown above the bar and those reported before

[Pandit et al. 2007; Razzaque et al. 2007; Ko et al. 2008] are shown below the bar. Green squares

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indicate families with NS. Orange squares indicate patients with LEOPARD syndrome and the yellow square indicates a patient with hypertrophic cardiomyopathy.
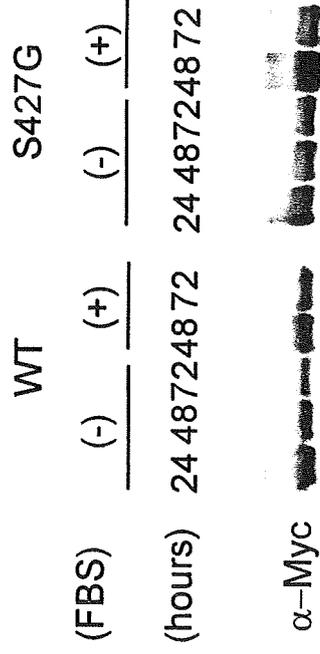
FIGURE 4. Schematic model of WT and mutant activation. A: In an inactive state, RAF1 is phosphorylated on S259 and S621 and is bound to 14-3-3. B: In growth-factor stimulation, the GTP-bound RAS binds to the CR1 domain of RAF1, which displaces 14-3-3. S259 is dephosphorylated by protein phosphatase 1 (PP1) and/or protein phosphatase 2A (PP2A). After RAF1 is recruited to the plasma membrane, phosphorylation of S338, Y341, T491 and S494 occurs. The phosphorylation of these residues is thought to be important for the full activation of RAF1. C: Mutants whose amino acid changes are located in the CR2 domain. It has been reported that S259 was phosphorylated by Akt and dephosphorylated by PP1 and/or PP2A. Amino acid changes in the CR2 domain would cause structural changes in the CR2 domain, leading to the access of PP2A to S259. Alternatively, Akt kinase would not be able to phosphorylate S259. S259 is dephosphorylated without stimulation and substrate(s) would be able to enter the kinase domain, leading to a partial activation. RBD, RAS-binding domain; CRD, cysteine-rich domain; KD, kinase domain; IH, isoform-specific hinge segment region
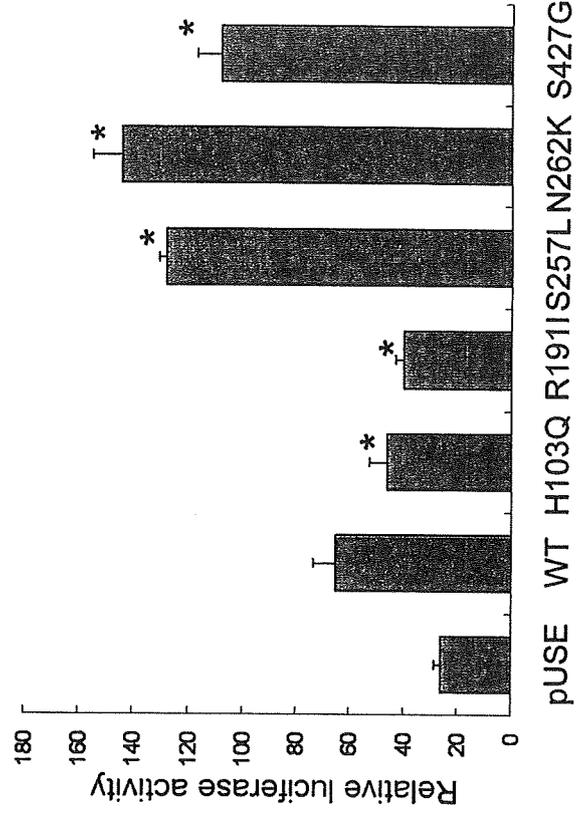
A

α-pS259
α-Myc
α-pERK
α-ERK

pUSE
WT
S257L
S259F
P261A
N262K
S427G
pUSE EGF(+)
Cell lysate

B

OD (405nm)

1.4
1.2
1
0.8
0.6
0.4
0.2
0

WT   WTpS   S257L   S257LpS   N262K   N262KpS

C

α-FLAG
α-Myc
IP:α-Myc

α-pS259
α-Myc
cell extracts

pUSE
WT
S257L, S621A
N262K, S621A

D

α-RAF1
α-FLAG
IP:α-FLAG

α-pS259
α-Myc
cell extracts

pUSE
WT
S257L, S621A
N262K, S621A

E

R191I (1)
S257L(11)
S259F (1)
P261A (1)/L (1)
N262K(1)   S427G (1)
R256S (1)
V263A (1)
D486N / T491I
G (2)
P261S / A / L (6)
T260R/ I(2)
S259F/T (2)
S257L (14)
L613V (3)
/R (2)
S612T (1)
L613V (1)

Table 1. *RAF1* mutations identified in this study

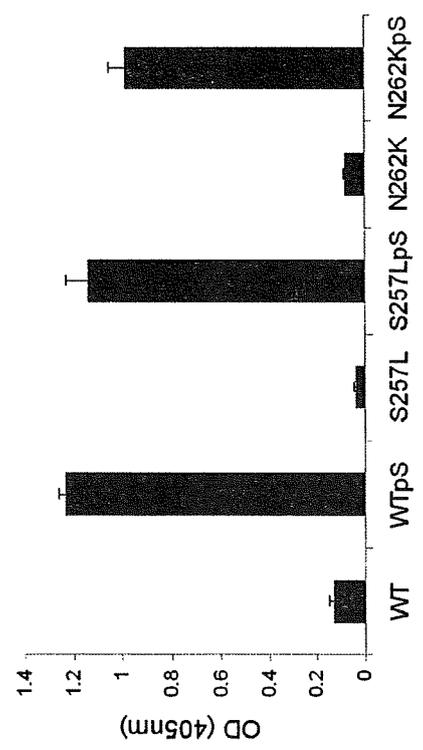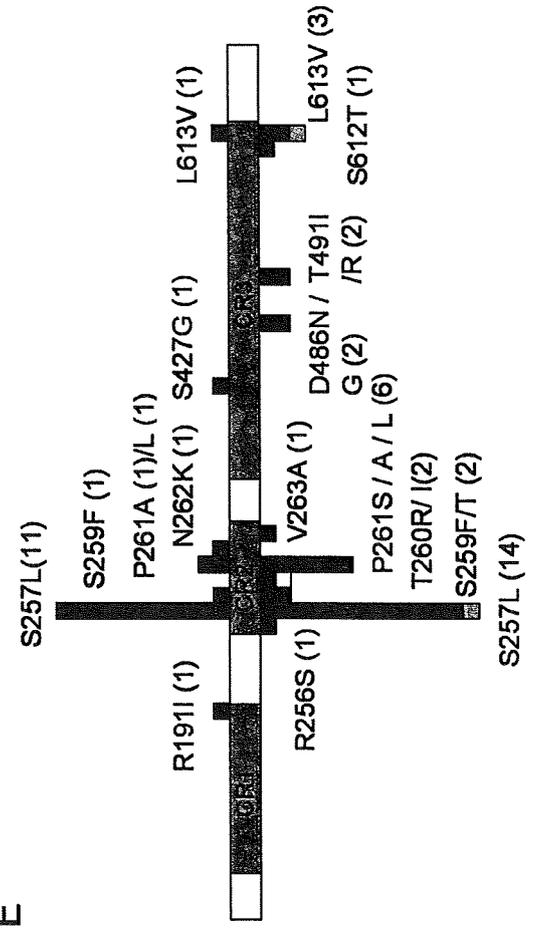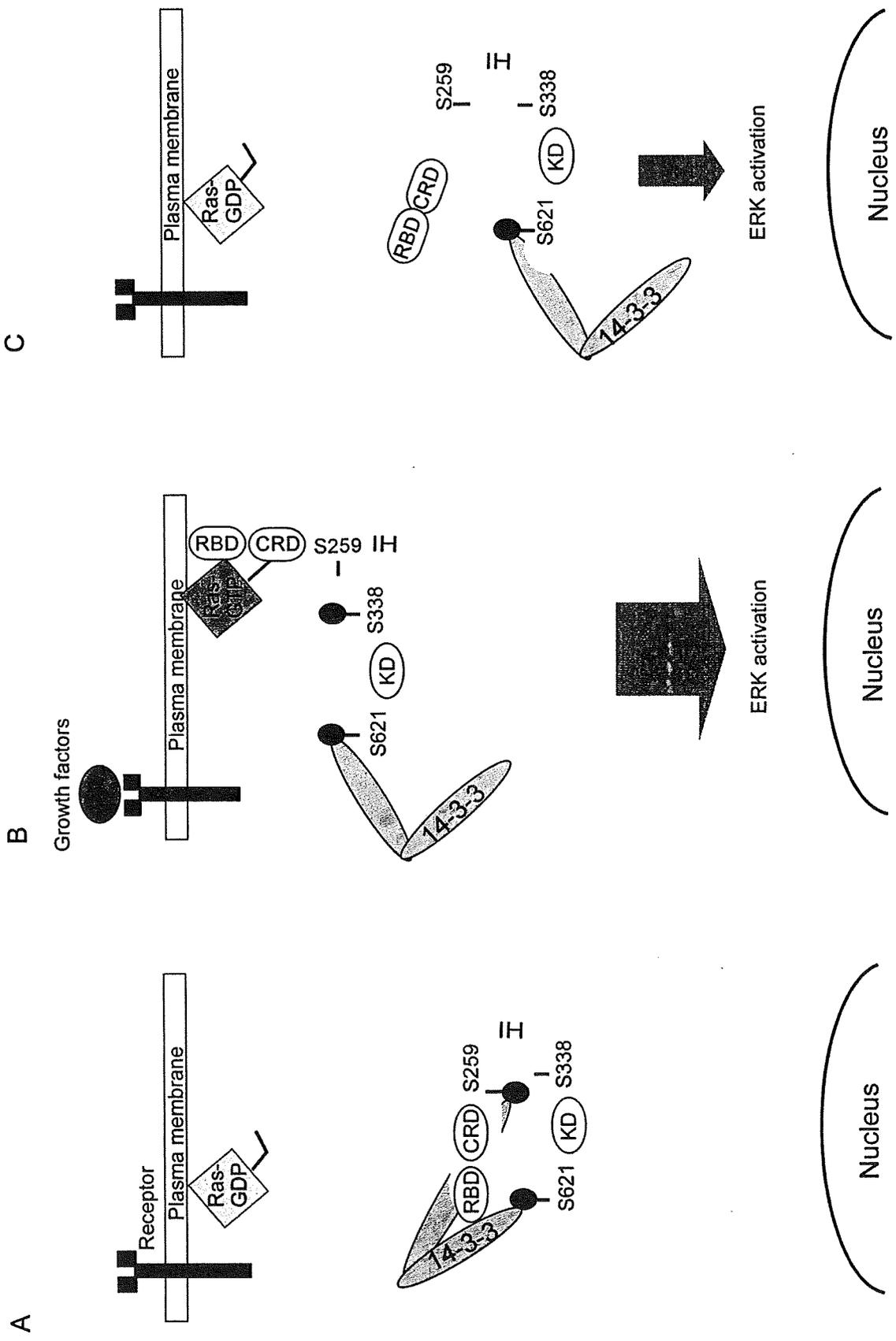| Patient ID	Country of origin	Final diagnosis	Exon	Nucleotide Change	Amino acid change	Domain	Genotype of father/mother
NS213	France	atypical NS	5	c. 572G>T	p.R191I[a]	CR1	NA
NS39	Japan	NS	7	c.770C>T	p.S257L	CR2	NA
NS86	France	NS	3, 7	c.309C>G c.770C>T	p.H103Q p. S257L	CR1, CR2	H103Q/WT WT/WT
NS92	Germany	NS	7	c.770C>T	p.S257L	CR2	WT/WT
NS135	Japan	NS	7	c.770C>T	p.S257L	CR2	NA
NS146	Spain	NS	7	c.770C>T	p.S257L	CR2	NA
NS199	Japan	NS	7	c.770C>T	p.S257L	CR2	NA
NS200	France	NS	7	c.770C>T	p.S257L	CR2	NA
NS215	Japan	NS	7	c.770C>T	p.S257L	CR2	NA
NS227	Japan	NS	7	c.770C>T	p.S257L	CR2	NA
NS256	Japan	NS	7	c.770C>T	p.S257L	CR2	NA
NS258	Japan	NS	7	c.770C>T	p.S257L	CR2	WT/WT
NS279	Japan	NS	7	c.776C>T	p.S259F	CR2	NA
NS210	France	NS	7	c.781C>G	p.P261A	CR2	WT/WT
NS205	France	CS[b]	7	c.782C>T	p.P261L	CR2	NA
NS209	France	CS[c]	7	c.786T>A	p.N262K[a]	CR2	WT/WT
NS222	Japan	NS	12	c.1279A>G	p.S427G[d]	CR3	WT/p.S257L
NS285	Japan	NS	17	c.1837C>G	p.L613V	CR3	NA

NS, Noonan syndrome; CS, Costello syndrome; WT, wild-type; CR, conserved-region; NA, not available

[a]Novel mutation

[b]Detailed clinical manifestations were not obtained.

[c]The patient died at one month.

[d] The mutation was previously identified in a patient with a therapy-related acute leukemia.

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Table 2. Clinical manifestations in *RAF1*-positive patients in this study and past studies

	Present cohort (%)	NS with *RAF1* mutations (%)	LS with *RAF1* mutations (%)
Number of patients in total	17	35[a]	2
Perinatal abnormality			
Polyhydramnios	6/15 (40)	6/19 (32)	ND
Fetal macrosomia	5/11 (45)	6/20 (30)	ND
Growth and development			
Growth failure[b]	10/12(83)	3	ND
Mental retardation	6/11 (55)	19/34 (56)	1
Outcome			
Died	4/17 (24)	2/11 (18)	ND
Craniofacial characteristics			
Relative macrocephaly	16/17 (94)	16/21 (76)	ND
Hypertelorism	14/15 (93)	20/21 (95)	2
Downslanting palpebral fissures	10/16 (63)	19/21 (90)	2
Ptosis	9/16 (56)	19/21 (90)	1
Epicanthal folds	12/14 (86)	12/21 (57)	1
Low-set ears	14/15 (93)	18/21 (86)	2
Skeletal characteristics			
Short stature	11/15 (73)	30/35 (86)	2
Short neck	14/15 (93)	21/31 (68)	2
Webbing of neck	13/16 (81)	25/30 (83)	2
Cardiac defects			
Hypertrophic cardiomyopathy	10/16 (63)	27/35 (77)	2
Atrial septal defect	5/16 (31)	11/35 (31)	0
Ventricular septal defect	3/17 (18)	3/35 (9)	0
Pulmonic stenosis	7/15 (47)	4/35 (11)	1
Patent ductus arteriosus	2/17 (12)	ND	ND
Mitral valve anomaly	5/17 (29)	8/32 (25)	2
Arrhythmia	6/16 (38)	8/9 (89)	ND
Others	TR 1, PH 1, atrioventricular valve dysplasia 1,	polyvalvular dysplasia 2 pulmonary valve	

1

	valvular AS 1	dysplasia 1, PFO 1, TOF 2, AS 1, right shaft deflection 1	
Skeletal/Extremity deformity			
Cubitus valgus	2/9 (22)	7/22 (32)	2
Pectus deformity	5/13 (38)	20/31 (65)	2
Others		prominent finger pads 2	prominent finger pads 1
Skin/Hair anomaly			
Curly hair	8/17 (47)	6/24 (25)	2
Hyperelastic skin	7/12 (58)	5/21 (24)	2
Café au lait spots	1/14 (7)	2/20 (10)	2
Lentigines	1/14 (7)	2/21 (10)	2
Naevus	3/15 (20)	9/22 (41)	0
Others	low posterior implantation 4, hyperpigmentation 3, redundant skin 3, sparse hair 2, sparse eyebrows 2, hemangioma 2	dry skin 3, sparse hair 3, sparse eyebrows 2, keratosis pilaris 2	
Genitalia	6/11 (55)	11/16 (69)	
Cryptorchidism	5/10 (50)	8/13 (62)	ND
Blood test abnormality			
Coagulation defects	2/11 (18)	1/4 (25)	ND

NS, Noonan syndrome; LS, LEOPARD syndrome; ND, not described; TR, tricuspid regurgitation; PH, pulmonary hypertension; AS, aortic stenosis; PFO, patent foramen ovale; TOF, tetralogy of Fallot
[a] Includes affected family members. Clinical manifestations in 21, 11 and 3 NS patients with *RAF1* mutations were summarized from three reports [Pandit et al. 2007], [Razzaque et al. 2007], [Ko et al. 2008], respectively.
[b] Includes poor weight gain, postnatal failure to thrive, poor feeding in infancy and developmental delay

Table 3. Clinical manifestations in NS patients with *RAF1*, *PTPN11*, *SOS1* and *KRAS* mutations

	RAF1[a] (%)	PTPN11[b] (%)	SOS1[c] (%)	KRAS[d] (%)
Total patients	52	172	73	18
Perinatal abnormality				
Polyhydramnios	12/34 (35)	ND	9/16 (56)	2
Fetal macrosomia	11/31 (35)	ND	9/15 (60)	ND
Growth and development				
Growth failure[e]	13/15 (87)	poor feeding in infancy 35/56 (63)	ND	3/3 (100)
Mental retardation	25/45 (56)	71/164 (43)	12/67 (18)[f]	16/17 (94)[g]
Outcome				
Died	6/28 (21)	ND	ND	ND
Craniofacial characteristics				
Relative macrocephaly	32/38 (84)	ND	9/21 (43)[f]	9/11 (82)
Hypertelorism	34/36 (94)	15/28 (54)[f]	5/6 (83)	12/12 (100)
Downslanting palpebral fissures	29/37 (78)	19/28 (68)	20/22 (91)	9/12 (75)
Ptosis	28/37 (76)	18/29 (62)	19/24 (79)	10/15 (67)
Epicanthal folds	24/35 (69)	15/28 (54)	ND	2/9 (22)[f]
Low set ears	32/36 (89)	56/64 (88)	20/22 (91)	7/10 (70)
Skeletal characteristics				
Short stature	41/50 (82)	97/172 (56)[f]	22/58 (38)[f]	12/17 (71)
Short neck	35/46 (76)	15/29 (52)[f]	17/22 (77)	9/10 (90)
Webbing of neck	38/46 (83)	36/122 (30)[f]	3/6 (50)	7/14 (50)[f]

1

Cardiac defects

Hypertrophic cardiomyopathy	37/51 (73)	10/135 (7)[f]	7/73 (10)[f]	3/18 (17)[f]
Septal defect	22/52 (42)	41/170 (24)[f]	17/73 (23)[f]	5/18 (28)
Atrial septal defect	16/51 (31)			4/18 (22)
Ventricular septal defect	6/52 (12)			1/18 (6)
Pulmonic stenosis	11/50 (22)	125/171 (73)[g]	53/73 (73)[g]	7/18 (39)
Patent ductus arteriosus	2/20 (10)	ND	ND	1/18 (6)
Mitral valve anomaly	13/49 (27)	ND	ND	3/18 (17)
Arrhythmia	14/25 (56)	ND	ND	ND

Skeletal/Extremity deformity

Cubitus valgus	9/31(29)	14/61 (23)	1/6 (17)	2/2 (100)
Pectus deformity	25/44 (57)	108/171 (63)	38/56 (68)	13/16 (81)

Skin/Hair anomaly

Curly hair	14/41 (34)	ND	15/22 (68)[g]	1/12 (8)
Hyperelastic skin	12/33 (36)	ND	1/6 (17)	3/12 (25)
Café au lait spots	3/34 (9)	ND	1/6 (17)	1/9 (11)
Lentigines	3/35 (9)	ND	ND	ND
Naevus	12/37 (32)	ND	ND	ND

Genitalia

Cryptorchidism	13/23 (57)	75/138 (54)	22/39 (56)	4/11 (36)

Blood test abnormality

Coagulation defects	3/15 (20)	46/90 (51)	14/66 (21)	2/9 (22)

ND, not described

[a][Pandit et al. 2007; Razzaque et al. 2007; Ko et al. 2008] and this study.

[b][Tartaglia et al. 2002; Musante et al. 2003; Zenker et al. 2004; Jongmans et al. 2005]

[c] [Roberts et al. 2007; Tartaglia et al. 2007; Zenker et al. 2007a; Ferrero et al. 2008; Narumi et al.

2008;Ko et al. 2008]

[d] [Carta et al. 2006; Schubbert et al. 2006; Zenker et al. 2007b; Lo et al. 2008;Ko et al. 2008]

[e] Includes poor weight gain, postnatal failure to thrive, poor feeding in infancy and developmental delay

[f] The frequency of the manifestation in patients with the gene was significantly lower compared with that observed in *RAF1*-positive patients (P<0.05 by Fisher's exact test.).

[g] The frequency of the manifestation in patients with the gene was significantly higher compared with that observed in *RAF1*-positive patients (P<0.05 by Fisher's exact test.).

Premature ovarian failure and androgen receptor gene CAG repeat lengths weighted by X chromosome inactivation patterns

The CAG repeat lengths weighted by X-inactivation ratios were significantly shorter in 58 Japanese patients with premature ovarian failure (POF) than in 42 age-matched control females with normal menses. The results suggest that short CAG repeats with a relatively high androgen receptor function may constitute a susceptibility factor for the development of POF. (Fertil Steril® 2009;91:649–52. ©2009 by American Society for Reproductive Medicine.)

Premature ovarian failure (POF) is a heterogeneous condition defined by the triad of primary or secondary amenorrhea, hypergonadotropism, and hypoestrinism in females less than 40 years old (1). While POF is frequently observed in females with sex chromosome aberrations, it also occurs in females with normal karyotypes (1). Although underlying factors for POF have been poorly elucidated in females with normal karyotypes, various genetic and environmental factors have been implicated in the development of POF. For example, mutations of several genes such as *BMP15*, *FOXL2*, and *NOBOX* as well as premutations of *FMR1* are known to cause POF (2–5), and several candidate genes such as *LHX8* and *GDF9* have been identified (6). Furthermore, chemotherapy, radiation, and autoimmune dysfunction also constitute risk factors for POF (1).

The androgen receptor (AR) plays a crucial role in sex development by mediating the biological effects of androgens (7). The *AR* gene resides on Xq12 and is made up of eight exons. Exon 1 harbors a highly polymorphic CAG repeat encoding a polyglutamine tract, and functional studies with different CAG repeat numbers have indicated an inverse relationship between the CAG repeat number and the transactivation function of *AR* (7). Consistent with this, the CAG repeat polymorphism is known to constitute a susceptibility factor for various androgen-related diseases in males (7). For example, while both positive and negative results have been reported, overall data from a large number of association studies argue that the CAG repeats tend to be long in males with undermasculinized genitalia and

spermatogenic dysfunction and short in those with prostate cancers (7–9).

Similar association studies have also been performed in females with hirsutism and polycystic ovary syndrome (PCOS) together with X-inactivation analysis, revealing both positive and negative results (10–14). This would not necessarily be inconsistent with the CAG repeat polymorphism functioning as a susceptibility factor for androgen-related diseases in females as well as in males because the susceptibility effect may be detected in some patient groups but not in other patient groups. However, the data remain scanty, and further studies are necessary to draw a certain conclusion as to whether the CAG repeat polymorphism forms a susceptibility factor for androgen-related disorders in females. Thus, we performed CAG repeat length and X-inactivation analyses in POF patients because ovarian function is subject to androgen effects (1).

We studied 58 Japanese patients with POF. The menarcheal age ranged from 10 to 15 years (mean ± SD, 12.7 ± 1.2 years; menarcheal age in normal Japanese girls, 12.3 ± 1.3 years), and the age of POF onset (amenorrhea persisting ≥ 6 months) ranged from 13 to 39 years (median, 30 years). At the first medical examination, serum FSH was 44–245 IU/L (median, 94 IU/L), LH was 6–70 IU/L (median, 28 IU/L), and elevated FSH was repeatedly observed. Serum E_2 was undetectable in 45 patients and ranged from 10 to 72 pg/mL (35 to 250 pmol/L) in 13 patients. Serum T was not measured.

All 58 patients satisfied the following criteria: [1] lack of somatic abnormalities, [2] absence of clinically discernible autoimmune diseases, [3] no history of chemotherapy or radiation, [4] 46,XX karyotype in all the ≥ 30 lymphocytes examined, [5] no demonstrable mutations in the coding regions of *BMP15* and *GDF9*, and [6] no *FMR1* premutation. Two patients were familial cases with a similarly affected sister and/or mother, and the remaining 56 patients were sporadic cases. For controls, DNA samples from 42 Japanese females with proven fertility and normal menses aged 22–45 years (median, 34 years) were obtained from

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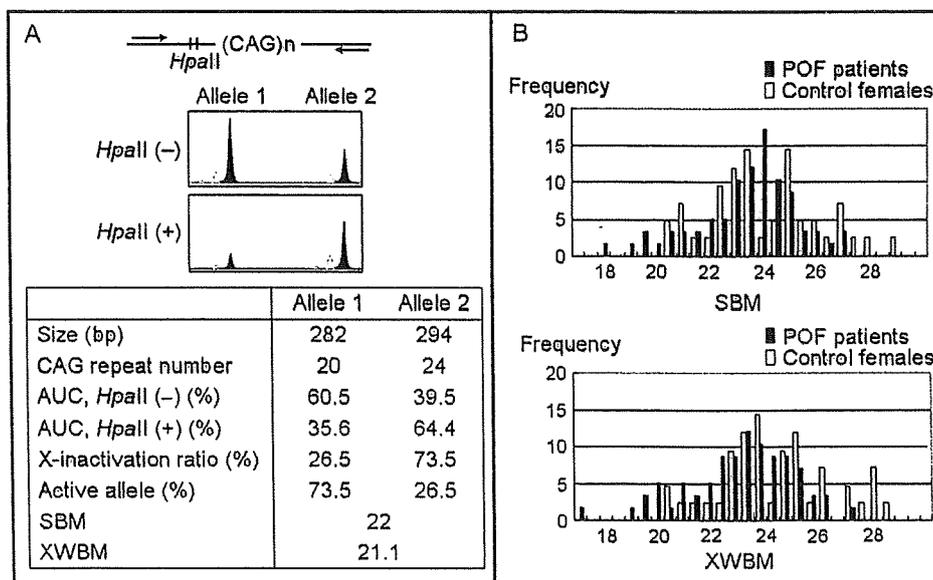
the Japanese Collection of Research Bioresources and similarly analyzed with permission. This study was approved by the Institutional Review Board committees of the investigators' affiliations. There is no conflict of interest.

CAG repeat length and X-inactivation analyses were performed by the previously reported method (15), with some modifications. In brief, leukocyte genomic DNA was polymerase chain reaction (PCR) amplified with a fluorescent labeled forward primer and an unlabeled reverse primer flanking the CAG repeat region and the two methylation sensitive *HpaII* sites at exon 1 of *AR*, before and after *HpaII* digestion (Fig. 1). The primer sequences and the PCR conditions were as described elsewhere (15). PCR products were obtained from both active and inactive X chromosomes before *HpaII* digestion and from inactive X chromosomes alone after *HpaII* digestion. For the CAG repeat

length analysis, the PCR products obtained before *HpaII* digestion were determined for size on an ABI PRISM 3100 autosequencer using GeneScan (Applied Biosystems, Norwalk, CT). Furthermore, to confirm the precise CAG repeat number, 12 PCR products of different sizes on GeneScan were subjected to direct sequencing on the autosequencer. For the X-inactivation analysis, the PCR products obtained before and after *HpaII* digestion were examined for area under curve on the autosequencer. The X-inactivation ratio was calculated using the area under curve after compensation for unequal amplification of the two alleles caused by the difference in the product size. The CAG repeat number of each subject was obtained as the simple biallelic mean (SBM) and as the X-weighted biallelic mean (XWBM). The XWBM was calculated using the X-inactivation ratio and was expressed as a rounded number by increments of 0.5.

FIGURE 1

CAG repeat length and X-inactivation analyses. (A) Representative results. PCR amplification has been performed with a fluorescent labeled forward primer and an unlabeled reverse primer (arrows) flanking the CAG repeat region and the two methylation sensitive *Hpa*II sites at exon 1 of *AR*. Before *Hpa*II digestion, two alleles have been delineated on the autosequencer; allele 1 is 282 bp long and contains 20 CAG repeats, and allele 2 is 294 bp long and contains 24 CAG repeats. The difference in the area under curve (AUC) between the two alleles is primarily due to the short allele being more easily amplified than the long allele. The small 279 and 291 bp peaks are by-products caused by the slippage phenomenon. After *Hpa*II digestion, the two alleles have been detected, and the difference in the AUC pattern before and after the *Hpa*II digestion is primarily caused by noneven X-inactivation. The X-inactivation ratio, which is a mirror image of the active allele ratio, is calculated using the AUCs before and after *Hpa*II digestion. In this patient, the allele 2 is more preferentially inactivated than the allele 1, and the allele 1 and the allele 2 are expressed in 73.5% and 26.5% of leukocytes, respectively. Thus, the SBM is obtained as 22, and the XWBM is calculated as 21.1. (B) Distribution of the SBMs and the XWBMs in patients with POF and control females. The XWBM has been obtained as a rounded number by increments of 0.5; for example, calculated XWBM values from 22.75 to 23.24 have been rounded as 23, and those from 23.25 to 23.74 have been rounded as 23.5.

	Allele 1	Allele 2
Size (bp)	282	294
CAG repeat number	20	24
AUC, *Hpa*II (−) (%)	60.5	39.5
AUC, *Hpa*II (+) (%)	35.6	64.4
X-inactivation ratio (%)	26.5	73.5
Active allele (%)	73.5	26.5
SBM	22	
XWBM	21.1	

Sugawa. POF and AR CAG repeat polymorphism. Fertil Steril 2009.

Representative results and the distributions of the SBMs and the XWBMs are shown in Figure 1. The SBMs and the XWBMs were found to follow the normal distribution in both the POF patients and the control females by the χ^2-test, and the variances were shown to be similar between the two groups by the F-test. Thus, the Student's t-test was employed for the statistical analysis, showing that the SBMs were comparable between the POF patients and the control females (mean ± SD, 23.3 ± 2.0 vs. 24.1 ± 2.1; $P=.07$), whereas the XWBMs were mildly but significantly shorter in the POF patients than in the control females (mean ± SD, 23.2 ± 2.1 vs. 24.2 ± 2.2; $P=.02$). Neither the SBM nor the XWBM was found to be correlated with the menarcheal age ($r = -0.02$; $P=.90$), the age of POF onset ($r = 0.08$, $P=.58$), the serum FSH value ($r = 0.01$, $P=.94$), and the LH value ($r = -0.05$, $P=.78$) by the Spearman's p test.

The XWBM was mildly but significantly shorter in the patients with POF than in the control females, although the SBM was comparable between the two groups of subjects. In this context, while the AR function has not been compared between the two groups of subjects in this study, the previous studies have indicated an inverse relationship between the CAG repeat number and the AR function (7). Thus, a relatively high AR function in somatic cells may be a susceptibility factor for the development of POF because the AR function in somatic cells would be better reflected by the XWBM than by the SBM. Since AR is clearly expressed in the granulosa cells of developing follicles (16), increased AR function may affect the follicular cell function, facilitating the development of POF. Indeed, androgen excess in several conditions such as 21-hydroxylase deficiency and PCOS is known to impair ovarian function (1, 17), although there has been no report documenting the relationship between androgen excess and POF. One may argue that POF can also result from dysfunction of oocytes in which the AR function would simply be reflected by the SBM rather than the XWBM because the two X chromosomes remain active in oocytes (18). However, the relevance of an oocyte factor to POF is unlikely in terms of the AR function because AR is not expressed in oocytes (16).

The SBM and the XWBM were not correlated with the menarcheal and POF onset ages or the serum gonadotropin values. This would at least in part be due to variations in genetic and environmental factors influencing menarcheal and menopausal ages and hormonal values.

Several points should be made with respect to the present study. First, most of the control females were less than 40 years of age. This may have affected the results of this study because some of them may develop POF at a later age. Second, the X-inactivation pattern was examined for leukocytes in this study as well as in the previous studies of the CAG repeat polymorphism in females (10–14). Thus, although the X-inactivation ratio is similar among different tissues in most individuals (19), the XWBM may more or less be different between leukocytes and target tissues

such as ovarian cells. Third, it remains to be examined whether CAG repeats tend to be short in other POF patients as well. Furthermore, POF may actually be associated with long CAG repeats with a relatively low AR function in ovarian follicular cells because POF is exhibited by female mice lacking AR (20). Thus, further studies are obviously necessary to examine the notion that short CAG repeats constitute a susceptibility factor for the development of POF.

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