Submicroscopic deletions at 16p13.3 in Rubinstein-Taybi syndrome: frequency and clinical manifestations in a North American population

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Abstract

Rubinstein-Taybi syndrome (RTS) is a well delineated multiple congenital anomaly syndrome characterised by mental retardation, broad thumbs and toes, short stature, and specific facial features. The recent localisation of the disorder to 16p13.3 and subsequent identification of a submicroscopic deletion of this region in RTS patients led us to screen a large cohort of affected subjects using the RT1 probe. Among 64 patients with clinical evidence of RTS, seven (11%) had a deletion. Another patient had a translocation of the region without evidence of a deletion. The features of coloboma, growth retardation, naevus flammeus, and hypotonia have a positive predictive value for the presence of an RT1 deletion. Because of the relatively low frequency of deletions in RTS, the RT1 probe is useful in diagnostic confirmation, but has limited use as a screening tool.
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Originally described in 1963, Rubinstein-Taybi syndrome (RTS) is a clinical diagnosis of mental retardation, short stature, broad thumbs and toes, and characteristic facial features.[1] Although the specific aetiology is unknown, there is evidence for a genetic basis. Several reports of parent to child transmission with a mild phenotype in the parent suggest a dominant trait, although the diagnosis in these cases has been questioned.[2 3] A breakthrough in the search for a specific aetiology in RTS occurred with the identification of cytogenetic rearrangements involving 16p13.3 in a few patients with classical features of RTS.[4–6] This led to the creation of the cosmid RT1 (D16S237) which hybridises to a region within 16p13.3 encompassing the 3' end of the CBP (cyclic AMP responsive binding (CREB) protein) gene. This protein is ubiquitously expressed as a coactivator in cyclic AMP regulated gene expression.[7] Using the RT1 probe, Breuning *et al*[8] identified a deletion of this region in six out of 24 RTS patients screened. Masuno *et al*[9] found an RT1 deletion in one out of 25 RTS patients in Japan. McGaughan *et al*[10] identified two deletion patients in a group of 16 British RTS patients. To date, 65 RTS patients have been screened with the cosmid RT1 (D16S237) probe with nine deletions identified.

We describe the results using the RT1 probe to screen a North American cohort of 64 RTS patients in order to establish the deletion frequency in this population, to identify clinical differences between deleted and non-deleted patients, and to test the hypothesis that patients with deletions may be more severely affected than those without deletions.

Methods

ASCERTAINMENT

Sixty-four patients with the diagnosis of RTS were ascertained through the Rubinstein-Taybi Parent Group (47 (73%)) and referrals from other genetic centres (17 (27%)). Clinical data, photographs, and medical records were collected on each patient to confirm the diagnosis of RTS. Thirty-nine specific traits, including growth parameters, were assessed for each patient. Since RTS is a short stature syndrome, microcephaly in this context was used as 1 SD below the 50th centile height age to assess true microcephaly in relation to body size and not small head size resulting from overall growth deficiency. Clinical assessment was made before knowledge of deletion status to minimise potential bias and to test the hypothesis that there are clinically distinguishable differences between deletion and non-deletion patients.

CYTOGENETIC STUDIES

Peripheral lymphocytes were cultured for 72 hours using conventional methods. Karyotyping with GTG banding was performed at the 550 to 750 band level. A minimum of five metaphase preparations were examined from each patient.

MOLECULAR STUDIES

The RT1 cosmid (D16S237), approximately 56 kb in size, was obtained in an *E coli* MC 1046 host. The presence of the probe was confirmed by digestion with *Eco*RI, which yielded a specific 5.2 kb fragment. The cosmid was

Table 1 Clinical features of Rubinstein-Taybi patients

	Current study		Stevens et al[15]
	Deletion patients (%)	Non-deletion patients (%)	RTS patients (%)
Craniofacial			
Curved nose	3/7 (43)	43/57 (75)	
Grimacing smile	5/7 (71)	29/57 (51)	
Epicanthic folds	4/7 (57)	28/57 (50)	
Downward slanting palpebral fissures	4/7 (57)	39/57 (68)	
Micrognathia	5/7 (71)	31/57 (54)	
Heavy arched eyebrows	5/7 (71)	24/57 (42)	
Ptosis	3/7 (43)	15/57 (26)	20/49 (40)
Long eyelashes	3/7 (43)	27/57 (47)	
Puffy face in infancy	3/7 (43)	20/57 (35)	
Ear anomalies	3/7 (43)	37/57 (33)	
Prominent forehead	2/7 (28)	19/57 (33)	
Ophthalmological			
Strabismus	2/7 (28)	22/57 (38)	24/50 (48)
Refractive error	1/7 (14)	23/57 (40)	19/50 (38)
Coloboma	3/7 (43)	3/57 (5)	3/49 (6)
Cataracts	0/7 (0)	2/57 (4)	
Hypertelorism	1/7 (14)	14/57 (24)	
Cardiac			
Congenital heart defects	2/7 (28)	23/57 (40)	19/50 (380
Orthopaedic			
Stiff unsteady gait	4/5 (80)	27/54 (50)	38/50 (76)
Broad thumbs	7/7 (100)	57/57 (100)	50/50 (100)
Broad big toe	6/7 (85)	49/57 (86)	50/50 (100)
Other broad fingers	2/7 (28)	19/57 (33)	
Polydactyly	1/7 (14)	5/57 (9)	5/50 (10)
Syndactyly	0/7 (0)	4/57 (7)	
Clinodactyly	1/7 (14)	13/57 (23)	
Radial deviation of thumb	3/7 (43)	23/57 (38)	19/50 (38)
Sternal abnormalities	1/7 (14)	11/57 (19)	
Neurological			
Hypotonia	5/7 (71)	27/57 (43)	
Feeding difficulties	3/7 (28)	20/57 (35)	
Seizures	1/7 (14)	5/57 (8)	
Structural CNS abnormalities	0/7 (0)	2/57 (4)	
Microcephaly	5/7 (71)	30/57 (53)	
Growth retardation	7/7 (100)	35/51 (68)	
Developmental delay	7/7 (100)	57/57 (100)	
Dermatological			
Hypertrichosis	4/7 (57)	21/57 (37)	
Naevus flammeus	4/7 (57)	15/57 (26)	
Urological			
Cryptorchidism	5/6 (83)	21/33 (64)	21/21 (100)
Renal abnormalities	1/7 (14)	2/57 (4)	
Endocrinological			
Delayed bone age	1/7 (14)	11/57 (19)	
Birth weight (average)	2485 g	3019 g	3090 g

Other features noted in deletion patients include apnoea, autism, and neuroblastoma and IgG subclass deficiency.
Other features in non-deletion patients include apnoea, depression, bipolar disorder, schizoid personality, supernumerary teeth, ulcerative colitis, IgG subclass deficiency, and supernumerary ribs.

labelled using the BioNick Labelling System from Life Technologies Inc under conditions optimised for biotin. The labelling reaction was verified by the presence of a 300-400 base pair band on agarose gel. A 7-8 μg aliquot of labelled RT1 cosmid was mixed with 500 μg Human Cot DNA and 500 μg salmon sperm DNA from Gibco BRL Inc as non-specific blocking agents for a final volume of 150 μl. Fluorescent in situ hybridisation was performed on metaphase preparations under standard conditions using a mixture of biotin labelled RT1 probe and digoxigenin labelled α satellite probe for chromosome 16 from Oncor Inc. Two microlitres of the RT1 probe and 0.75 μl of the 16 α satellite probe were added to 30 μl Hybrisol VI and denatured at 70°C for five minutes before an overnight hybridisation. Detection and DAPI (6-diamidino-2-phenylindole) staining for dual colour were carried out according to standard protocol. Ten to 15 metaphases were evaluated on each patient.

STATISTICAL METHODS

The presence or absence of 39 clinically discernible traits was scored on each patient. Multiple logistic regression with a stepwise computed model using the True Epistat (trademark) computer program was used to analyse the data with presence or absence of a deletion as the outcome criteria. The data were analysed as a whole for overall differences, and a predictive model was constructed using traits with statistical significance of $p>0.10$ (chi-square).

Results

CLINICAL FINDINGS

There were 64 patients in total with 33 males and 31 females (clinical findings are summarised in table 1). The age range of patients was 2 weeks to 56 years. All patients had broad thumbs and distinctive facial features. Mental retardation was present in all those over 2 years of age where mental retardation could be accurately assessed. Short stature (height <3rd centile) was present in 48/64 (75%) of patients. Disproportionate microcephaly (head circumference more than 1 SD below height age) was present in 17/41 (41%) of patients. Congenital heart disease was present in 24/64 (39%) of patients, which parallels the previously reported incidence of congenital heart disease (38%) in RTS.[11]

CYTOGENETIC FINDINGS

Karyotype analysis showed a normal cytogenetic pattern at the 550 to 750 band level with structurally normal chromosome 16 homologues in 63/64 patients. A balanced translocation (1;16) was identified in one patient. The translocation is designated 46,XY,t(1;16)(p34.1;p13.2).

FLUORESCENT IN SITU HYBRIDISATION

Using the RT1 cosmid, there were seven deletions identified by the presence of only one RT1 signal and two 16 α satellite signals in a single patient, indicating a submicroscopic deletion of the RT1 region. The translocation patient showed two RT1 signals, one on chromosome 16 and the other on the derivative chromosome 1, indicating that the RT1 region was not deleted, but moved from its usual position by the translocation.

COMPARISON OF DELETION AND NON-DELETION PATIENTS

A summary of the clinical features of deletion and non-deletion patients is presented in table 1. The seven deletion patients had an age distribution of 2 weeks to 10 years with a mean age of 4.6 years. The sex distribution was six males and one female. The non-deletion patients ranged from 2 months to 56 years with a mean age of 11.7 years. Growth retardation was present in 7/7 (100%) of deletion patients and 35/51 (68%) of non-deletion patients. Disproportionate microcephaly was seen in 1/6 (16%) deletion patients and 16/35 (45%) non-deletion patients. Congenital heart defects were present in 2/7 (28%) deletion patients and 25/57 (40%) non-deletion patients. Broad toes

Table 2 Prediction of an RT1 deletion using selected Rubinstein-Taybi syndrome traits

Trait	Odds ratio	p value
Positive predictors		
Growth retardation	>100:1	0.10
Naevus flammeus	14:1	0.05
Coloboma	4.5:1	0.10
Hypotonia	3:1	0.10
Negative predictors		
Prominent forehead	0.15:1	0.20
Refractive error	0.12:1	0.10

Using these six traits as predictors of deletion status, sensitivity=87.5%, specificity=80%, false positive=61%, false negative=2.2%, correct classification=81%.

Table 3 Frequency of deletions of 16p13.3 in Rubinstein-Taybi syndrome

	Total No of patients	No of deletions	%
Breuning *et al*[8] (The Netherlands)	24	6	25
Masuno *et al*[9] (Japan)	25	1	4
McGaughan *et al*[10] (United Kingdom)	16	2	12.5
Present study (USA)	64	7	11
Total	125	16	12.8

were present in 6/7 (85%) deletion patients and 25/57 (40%) non-deletion patients. Average birth weight was 2845 g for deletion patients and 3019 g for non-deletion patients. The average number of the scored features was 18.4 in deleted patients and 16.7 in non-deleted patients.

STATISTICAL ANALYSIS

Growth retardation (height, weight, and head circumference <3rd centile), hypotonia, ocular coloboma, naevus flammeus, prominent forehead, and refractive error were the subset of traits with statistical significance. An RT1 deletion was more likely to be identified in patients with growth retardation (odds ratio >100:1), hypotonia (odds ratio 3:1), ocular coloboma (odds ratio 4.5:1), and naevus flammeus (odds ratio 14:1). The presence of prominent forehead and refractive error predicted non-deletion status with odds ratios of 0.15:1 and 0.12:1, respectively. Table 2 summarises the odds ratios and statistical significance of these traits as predictors of deletion status. Using these six traits to create a predictive model yielded a sensitivity of 87.5%, specificity of 80%, false positive rate of 61%, false negative rate of 2.2%, and correct classification of 81%. This model accounted for 38% of log likelihood probability of the presence of an RT1 deletion. The model chi-square was 18.3 with 6 degrees of freedom, p=0.005.

Discussion

Rubinstein-Taybi syndrome is a multisystem dysmorphic syndrome with many non-specific features, making diagnosis occasionally difficult. Mental retardation, broad thumbs, short stature, and characteristic facial features are minimal diagnostic criteria. Epicanthic folds, growth retardation, and arched eyebrows are part of RTS, yet are not pathognomonic. The availability of a molecular test would greatly aid the clinician in the confirmation of this diagnosis. The creation of the RT1 probe seemed to address this need.

Use of the RT1 probe has been reported in three small series of RTS patients before this series (table 3), primarily because of the rarity of patients with RTS. Our study, the first series in North America and the largest to date, doubles the number of RTS patients screened with the RT1 probe. The frequency of RT1 deletion in our group is 7/64 (11%), which is comparable to the pooled data from all studies, a deletion frequency of 12.8%. As a result of these data, the clinical use of the RT1 probe is limited, as more than 87% of clinically affected RTS patients would not be detected with this probe. A deletion, however, would still confirm the diagnosis.

The specific pathogenesis of the RTS phenotype is still not clear. The RT1 region contains the CBP (CREB binding protein) gene, a cyclic AMP binding protein. Protein truncation studies have identified abnormalities of this protein in a small number of subjects with RTS, suggesting that this gene is involved in the RTS phenotype.[12] The role of CBP in embryogenesis is currently unknown. The multiple malformations of RTS would suggest that its potential importance in morphogenesis bears further study.[13] However, since deletions have been found in only 12% of the RTS patients studied, different mechanisms producing the syndrome must be present. A point mutation within the CBP gene without deletion would not be detected by the RT1 probe and may be the mechanism in some patients. Uniparental disomy of chromosome 16 has been studied, but there is no evidence to support this as an aetiological mechanism.[14] Alternatively, genetic heterogeneity involving other loci is a possibility.

In general, our deleted patients do not seem clinically distinct from their non-deleted counterparts. Statistically, as a whole, they do not present a different phenotype. Our initial hypothesis that deleted patients might be more severely affected was not borne out in our population. This would support the notion of a specific critical locus and not a contiguous gene deletion syndrome.

Four features, all previously described in RTS (growth retardation, coloboma, naevus flammeus, and hypotonia), had a positive predictive value for the presence of a deletion. Two features of RTS patients (prominent forehead and refractive error) had a negative predictive value for presence of a deletion. While not conclusive, the presence or absence of these features may be helpful to clinicians. The benefit of establishing the presence of a coloboma may suggest obtaining ophthalmological consultation as a routine part of the RTS patient evaluation.

The RT1 probe may be useful for diagnostic confirmation of a diagnosis of RTS, but the low detection frequency makes its benefit as a screening test limited. Further molecular analysis of people who do not have an RT1

deletion is needed for clarification of the genetic aetiology of this syndrome.

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