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Molecular genetic analysis of mentally retarded males with features of the fragile-X syndrome

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Abstract

The fragile-X [fra(X)] or Martin-Bell syndrome is the most common familial cause of mental retardation and is characterized by the presence of an Xq27.3 chromosome fragile site. Unstable DNA sequences representing large increases in the number of CGG trinucleotide DNA base repeats of the FMR-1 gene are located at the fragile site and responsible for the fra(X) syndrome. In order to identify whether cytogenetically normal yet mentally retarded males without a known cause of their retardation had expansion of the CGG repeat segment of the FMR-1 gene, molecular genetic studies using Southern hybridization were performed with two DNA probes (fxa241 and Ox1.9) following digestion of genomic DNA from each patient with restriction enzymes PstI and EcoRI/EagI, respectively. DNA studies were performed on 20 (12.3%) out of 162 (122 white and 40 black people) cytogenetically normal mentally retarded males without a known cause of their retardation, but with high anthropometric discriminant values and/or clinical checklist scores identified previously and consistent with the fra(X) syndrome. None of the 20 males showed expansion of the CGG repeat of the FMR-1 gene detectable with the two probes used in this study. While heterogeneous single base pair substitutions, or small deletions or insertions in the FMR-1 gene could exist in our patients, aberrations in other X-linked mental retardation genes, not identified to date but whose gene product can produce a phenotype similar to fra(X), either independently or in conjunction with the recently identified FMR-1 protein, should be considered and are under investigation. Our study supports the idea that major FMR-1 gene expansion detectable with Southern hybridization is rare in cytogenetically normal mentally retarded males, including those with physical and behavioural features seen in the fra(X) syndrome.

Introduction

The fragile-X [fra(X)] or Martin-Bell syndrome is the most common familial cause of mental retardation, and is characterized by the presence of an Xq27.3 fragile site (gene symbol FRAXA) at the distal end of the long arm of the X-chromosome. Clinical features include mental retardation, macro-orchidism, large or prominent ears, a long narrow face, and mild connective tissue dysplasia (Chudley & Hagerman 1987; Hagerman 1987, 1992; Butler 1988; Butler *et al.* 1991a). The incidence of the fra(X) syndrome is approximately one in a thousand males (Herbst & Miller 1980; Turner *et al.* 1986; Webb *et al.* 1986).

Expression of the Xq27.3 site in 4% of male cells and in 2% of female cells grown in folate-deficient culture conditions is considered diagnostic for the fra(X) syndrome. The molecular basis of fra(X) syndrome has been attributed to the expansion of an unstable CGG trinucleotide DNA base repeats in the 5' untranslated region of the FMR-1 (familial mental retardation-1) gene located at the Xq27.3 fragile site (Yu *et al.* 1991; Pieretti *et al.* 1991; Pergolizzi *et al.* 1992; Knight *et al.* 1992; deVries *et al.* 1993; de Boule *et al.* 1993). Characterization of the FMR-1 gene and correlation of the clinical features with the size of the gene amplification are underway. When the size of the CGG repeat exceeds 200 repeats, the FMR-1 gene is methylated. This methylation results in the absence of the FMR-1 message and its encoded protein, FMRP, and its absence is believed responsible for the phenotype of the fra(X) syndrome (Ashley *et al.* 1993; Verheij *et al.* 1993). *In situ* hybridization with FMR-1 message RNA in normal individuals revealed widespread but not ubiquitous expression with abundant message present in testes and in neurons of the brain, which are regions of the body commonly affected in fra(X) syndrome individuals (Ashley *et al.* 1993). Alterations of the FMR-1 gene, detectable with several DNA probes isolated from the Xq27.3 fragile site using Southern hybridization, and more recently, with the newer polymerase chain reaction (PCR) methods are gaining acceptance for diagnostic and clinical applications.

Early recognition of fra(X) individuals can be difficult. Hence, Butler *et al.* (1991a, b, 1992, 1993) and Hagerman *et al.* (1991) reported certain physical characteristics (e.g. ear width, testicular volume, bizygomatic diameter, head breadth, plantar crease and hyperflexibility) useful in correctly classifying the fra(X) syndrome in greater than 90% of mentally retarded males.

A detailed anthropometric survey of 41 variables was reported on 110 mentally retarded males [39 with and 71 without the fra(X) syndrome] (Butler *et al.* 1991a), and significant differences were observed between fra(X) and non-fra(X) males for 18 of the measurements, with the greater measurements (excluding bizygomatic diameter) found in fra(X) males. Discriminant analysis of these patients was also performed and a discriminant function produced based on four of 30 anthropometric variables and age. The four variables in the discriminant function were the Z-scores representing testicular volume, ear width, bizygomatic diameter and head breadth. Patients with the fra(X) syndrome were distinguished from mentally retarded males without the fra(X) syndrome using the four variables at an overall correct classification rate of 95%.

In addition, discriminant analysis of variables from a 15 item clinical checklist and age in 188 mentally retarded males resulted in a discriminant function based on six variables (plantar crease, simian crease, hyperflexibility, large testes, large ears and a positive family history of mental retardation) (Butler *et al.* 1991b). Males with the fra(X) syndrome were distinguished by this analysis from mentally retarded males without the fra(X) syndrome at an overall correct classification rate of 93%.

Herein, we report molecular genetic analysis using two DNA probes and Southern hybridization for the CGG repeat segment of the FMR-1 gene with a relatively large number of cytogenetically normal mentally retarded males without a previously known cause of their

retardation, but possessing high anthropometric discriminant values and/or checklist scores consistent with the fra(X) syndrome, as described previously, in order to identify if these males had expansion of the CGG repeat segment of the FMR-1 gene as the cause of their retardation.

Materials and methods

Subjects

A detailed cytogenetic and clinical survey of 236 mentally retarded males [39 with and 197 without the fra(X) syndrome] was recently undertaken, and a comprehensive assessment of the cytogenetic, anthropometric and clinical findings was reported (Butler *et al.* 1990, 1991a, b, 1993; Butler & Singh 1993). Clinical descriptions and categories of mental retardation were previously reported for a subset of these individuals (201 of the 236 males) (Butler & Singh 1993). Because of the uncooperativeness of some patients or missing data (e.g. malformed or damaged external ears), anthropometric and cytogenetic data were only available for analysis on 162 (122 white and 40 black people) out of the 197 mentally retarded males with normal chromosomes, and 21 (17 white and 4 black) out of the 39 fra(X) males. The age range was 4–76 years for the 21 fra(X) males, with an average age of 21.3 ± 17.2 years. The fra(X) chromosome expression was $25 \pm 16\%$, with a range of 3–49%. The age range was 7–77 years for the 162 mentally retarded males with an average age of 40.0 ± 14.4 years.

All measurements and physical examinations were made by one of the authors (MGB) according to standard techniques as presented by Weiner & Lourie (1969) and Farkas (1981). Clinical examination included taking 31 anthropometric measurements (weight, height, eight linear, four breadth, 14 craniofacial, two skinfolds and testicular volume), recording of behavioural characteristics (e.g. tactile defensiveness, hand flapping and poor eye contact), and reviewing medical records as previously described (Butler *et al.* 1991a). The 15-item checklist (Butler *et al.* 1991b) had been completed previously for each individual. An item was scored as follows: (2) if it was definitely present; (1) if present in the past, questionably or borderline present; or (0) if never present.

Cytogenetics

Peripheral blood samples were previously collected and cultured for 96 h in both Medium 199 (a folate-deficient culture medium) supplemented with 5% foetal bovine serum and RPMI 1640 medium supplemented with 15% foetal bovine serum. Each medium was adjusted to a pH of 7.6. After 72 h, 5-fluoro-2'-deoxyuridine (FUDR) was added to the RPMI 1640 medium cultures to a final concentration of 10^{-7} M to enhance fragile site expression. Cells from both cultures were routinely harvested, stained with Giemsa and analysed for fragile sites (Butler *et al.* 1990). If fragile sites were observed, the chromosomes were destained then G-banded after trypsinization and the location of the fragile sites noted on the chromosomes. Seventy-five cells were studied for the appearance of the Xq27.3 fragile site from each patient (e.g. 50 cells from Medium 199 and 25 cells from RPMI 1640 medium), and three karyotypes or chromosome preparations from each

patient were analysed. The diagnosis of the fra(X) syndrome was made if 4% of the patient's cells were found to have the Xq27.3 fragile site.

DNA studies

High molecular weight DNA was extracted from peripheral blood leukocytes using standard procedures, as described elsewhere (Butler *et al.* 1993). Three to 5 µg of DNA was digested to completion in a double digest reaction containing both restriction endonucleases, *EcoRI* and *EagI*, for use with Ox1.9 DNA probe (kindly provided by Kay Davies), or in a single digestion with *PstI* for use with fxa241 DNA probe (Oncor Inc., Gaithersburg, MD, USA). The digested DNA was subjected to 0.8% agarose gel electrophoresis and DNA fragments were transferred to a nylon membrane, as previously described (Butler *et al.* 1993). Hybridization with either ³²P random prime radiolabeled Ox1.9 or fxa241 was performed for 48–60 h, excess unbound probe was removed and autoradiography was performed for 4–7 days at –70°C. DNA samples obtained from both a normal and fra(X) affected male were similarly processed and used as controls.

Results

Of the 201 mentally retarded males without a previously recognizable cause of their mental retardation surveyed in the present survey, 196 were normal including for the fra(X) syndrome, one patient had ring 22 syndrome and four patients were cytogenetically positive for the fra(X) syndrome. Additionally, 39 males with the fra(X) syndrome were available for analysis in the present study. Anthropometric measurements and checklist variables were available on 162 cytogenetically normal mentally retarded males and 21 fra(X) syndrome males expressing the Xq27.3 fragile site, as previously described (Butler *et al.* 1991a, b). The average discriminant value was 1.9 ± 1.5 (range –0.5–4.9) for the 21 fra(X) syndrome males calculated from the four anthropometric variables (testicular volume, ear width, bizygomatic diameter and head breadth), as previously described (Butler *et al.* 1991a) and -0.5 ± 1.0 (range –3.0–3.1) for the 162 cytogenetically normal mentally retarded males.

Table 1 shows summary data for anthropometric discriminant variables for the fra(X) syndrome and cytogenetically normal mentally retarded males utilized in this study. The average checklist score determined for the 21 fra(X) males, as previously described (Butler *et al.* 1991b), was 19.0 ± 5.4 (range 6–26) and 8.0 ± 4.0 (range 2–22) for the 162 cytogenetically normal mentally retarded males analysed in this study. Table 2 shows summary data for checklist variables from all fra(X) syndrome males and cytogenetically normal mentally retarded males utilized in this study.

In order to identify if cytogenetically normal yet mentally retarded males without a known cause of their retardation had expansion of the CGG repeat segment of the FMR-1 gene, Southern blot analysis was performed using two probes (fxa241 and Ox1.9) following digestion of genomic DNA with *PstI* and *EcoRI/EagI*, respectively. DNA studies were performed on 20 (12.3%) of the 162 cytogenetically normal mentally retarded males with high anthropometric discriminant values and/or checklist scores (Butler *et al.* 1991a, b).

Table 3 shows the clinical, anthropometric and discriminant data from the 20 cytogenetically normal males utilized in the DNA study. Table 4 shows the clinical and checklist variables, and scores for the 20 cytogenetically normal males utilized in this study. Nine of these individuals had no family history of mental retardation; five individuals (patients AB, KW, MN, WH and ED; see Table 4) had at least one first-degree relative (e.g. sibling or parent) with mental retardation, two individuals (patients EO and TB; see Table 4) had at least one second-degree relative (e.g. grandparent, uncle or aunt) with mental retardation, and the family history was unknown for four individuals (patients GF, GR, TL and LM; see Table 4). In several subjects, the discriminant value and/or checklist scores were consistent with the diagnosis of the fra(X) syndrome, and the cytogenetic and Southern blotting data were within normal range. None of the Southern blot autoradiograms of the 20 cytogenetically normal mentally retarded males with high discriminant values and/or checklist scores showed expansion of the CGG repeat of the FMR-1 gene with the two probes used in this study in either single or dual enzyme DNA digestions. Cytogenetically positive (>4% expression of the Xq27.3 site) males for the fra(X) syndrome showed abnormal DNA findings consistent with the diagnosis of the fra(X) syndrome (Fig. 1).

Discussion

We undertook a relatively large clinical, cytogenetic and molecular genetic survey of mentally retarded males without a previously recognizable cause of their mental retardation in order to identify if mentally retarded males with no known cause of their retardation, and with clinical and anthropometric findings consistent with the Martin-Bell phenotype but with normal cytogenetic studies, could have alterations of the FMR-1 gene. Two percent of the 201 mentally retarded males showed the fra(X) chromosome in at least 4% of their cells; one patient had ring chromosome 22 syndrome. The remaining 196 males were cytogenetically normal including for the fra(X) syndrome. DNA studies with Southern blot analysis were undertaken using two probes spanning the CGG repeat segment 5' to the FMR-1 gene of 20 males with clinical and anthropometric variables indicative of the fra(X) syndrome. However, none of the 20 males showed DNA alterations of the FMR-1 gene with the methodology described in this study.

A male with a typical Martin-Bell phenotype yet presenting with negative cytogenetic and DNA findings, similar to those males described here, has been described (Hirst *et al.* 1992). In addition, four fra(X) positive mentally retarded males with no expansion of the CGG repeat have been described (although sparse clinical and cytogenetic data were given) (Knight *et al.* 1992) and may represent other fragile sites in the Xq27 region. Although expansion of the CGG repeat is characteristic of most fra(X) affected males, other perturbations in the FMR-1 not detected by this methodology can exist (Wöhrle *et al.* 1992; de Boule *et al.* 1993; deVries *et al.* 1993) and may be present in the FMR-1 genes of some of the patients reported here. While heterogeneous single base pair substitutions, small deletions or insertions in FMR-1 could exist in our patients, aberrations in other X-linked mental retardation genes not identified to date, but whose gene product can either independently or in conjunction with the recently identified FMR-1 protein (Ashley *et al.* 1993; Verheij *et al.* 1993) produce a phenotype similar to fra(X), should be considered and are under investigation. Our study indicates that major FMR-1 gene expansion is rare in

cytogenetically normal mentally retarded males including those with physical and behavioural features seen in the fra(X) syndrome.

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Figure 1.

Representative Southern blot analysis with Ox1.9 probe of peripheral blood DNA digested simultaneously with *EcoRI* and *EagI* from cytogenetically normal mentally retarded males with clinical and anthropometric findings consistent with the Martin-Bell or fra(X) phenotype, an affected fra(X) chromosome positive male and an unaffected control male. Lanes: (1) DNA from a fra(X) syndrome male; (2–11) DNA from cytogenetically normal mentally retarded males with clinical and anthropometric findings (see Tables 3 & 4) consistent with the Martin-Bell or fra(X) phenotype (patients: 2=MN; 3=DB; 4=SC; 5=GR; 6=WS; 7=TL; 8=CM; 9=KW; 10=CP; 11=AB); (12) DNA from an unaffected control male. Arrows correspond to expansion of the CGG repeat in an affected fra(X) syndrome male (top) and 2.8 kb fragments consistent with a normal male (bottom).

Table 1

Summary data for anthropometric discriminant variables from all fragile-X syndrome males and cytogenetically normal mentally retarded males utilized in this study

Subject group	Age (years) ($\bar{X} \pm SD$); range (years)	Race	Fragile-X expression ($\bar{X} \pm SD$) (%)	Testicular volume (ml)($\bar{X}$ $\pm SD$)		Ear width (cm) ($\bar{X}$ $\pm SD$)		Bizygomatic diameter (cm) ($\bar{X} \pm SD$)		Head breadth (cm) ($\bar{X} \pm SD$)		Discriminant value ($\bar{X} \pm SD$); range		Checklist score ($\bar{X} \pm SD$); range	
				[Z score- $\bar{X}$ $\pm SD$]; range (ml)	[Z score- $\bar{X}$ $\pm SD$]; range (cm)	[Z score- $\bar{X}$ $\pm SD$]; range (cm)	[Z score- $\bar{X}$ $\pm SD$]; range (cm)	[Z score- $\bar{X}$ $\pm SD$]; range (cm)	[Z score- $\bar{X}$ $\pm SD$]; range (cm)	[Z score- $\bar{X}$ $\pm SD$]; range (cm)	[Z score- $\bar{X}$ $\pm SD$]; range (cm)				
Fragile-X (n = 21)	(21.3 $\pm$ 17.2) 4-76	17W:4B	25 $\pm$ 16	(34.6 $\pm$ 29.6) [10.2 $\pm$ 9.1] 2.4-100	(4.0 $\pm$ 0.4) [2.2 $\pm$ 1.8] 3.4-4.7	(12.5 $\pm$ 1.4) [-1.5 $\pm$ 1.6] 9.9-14.6	(14.7 $\pm$ 0.8) [-0.3 $\pm$ 1.2] 13.1-16	(1.9 $\pm$ 1.5) -0.5-4.9	(19.0 $\pm$ 5.4) 6-26						
Non-fragile-X (n = 162)	40 $\pm$ 14.4 7-77	122W:40B	0	(26 $\pm$ 13.1) [2.2 $\pm$ 2.7] 9-77	(3.8 $\pm$ 0.4) [1.1 $\pm$ 1.9] 3.0-5.0	(13.5 $\pm$ 0.9) [-0.7 $\pm$ 1.5] 9.0-15.0	(14.4 $\pm$ 0.8) [1.4 $\pm$ 1.4] 12.0-16.0	(-0.5 $\pm$ 1.0) -3.0-3.1	(8.0 $\pm$ 4.0) 2-22						

Summary data for checklist variables from all fragile-X syndrome males and cytogenetically normal mentally retarded males utilized in this study

Age (years) $\bar{X} \pm SD$, range	Race	Fragile-X expression (%)	MR [*] ($\bar{X} \pm SD$)	LE ($\bar{X} \pm SD$)	LT ($\bar{X} \pm SD$)	PC ($\bar{X} \pm SD$)	FH of MR ($\bar{X} \pm SD$)	HA ($\bar{X} \pm SD$)	TD ($\bar{X} \pm SD$)	SAS ($\bar{X} \pm SD$)	HE ($\bar{X} \pm SD$)	PS ($\bar{X} \pm SD$)	HF ($\bar{X} \pm SD$)	PBE ($\bar{X} \pm SD$)	SC ($\bar{X} \pm SD$)	HB ($\bar{X} \pm SD$)	PEC ($\bar{X} \pm SD$)	Checklist scores, range
Fragile-X (n = 21), [†] 11-17.2 4-76	17W-4B	25.1 $\pm$ 5.6	2-40	1.7 $\pm$ 0.7	1.8 $\pm$ 0.5	1.7 $\pm$ 0.7	1.6 $\pm$ 0.8	1.7 $\pm$ 0.7	1.2 $\pm$ 0.9	1.4 $\pm$ 0.8	1.2 $\pm$ 0.9	1.6 $\pm$ 0.6	1.2 $\pm$ 1.0	0.4 $\pm$ 0.8	0.8 $\pm$ 1.0	0.8 $\pm$ 0.9	0.5 $\pm$ 0.9	19.0-5.4 6-26
Non-Fragile-X (n = 162), [†] 40-14.4 7-77	122W-40B	0	2-20	1.0 $\pm$ 1.0	0.6 $\pm$ 0.8	0.3 $\pm$ 0.7	0.9 $\pm$ 1.0	0.6 $\pm$ 0.9	0.3 $\pm$ 0.7	0.4 $\pm$ 0.8	0.4 $\pm$ 0.8	0.1 $\pm$ 0.5	0.3 $\pm$ 0.7	0.1 $\pm$ 0.4	0.1 $\pm$ 0.4	0.5 $\pm$ 0.8	0.5 $\pm$ 0.8	8.0-4.0 2-22

^{*}MR, mental retardation; LE, large ears; LT, large testicles; PC, plantar crease; FH of MR, family history of mental retardation; HA, hyperactivity; TD, tactile defensiveness; SAS, short attention span; HE, hyperextensibility; PS, perseverative speech; HF, hand flapping; PBE, pale blue eyes; SC, simian crease; HB, hand biting; PEC, poor eye contact.

[†]Subject group

Clinical, anthropometric, discriminant values and checklist score data of cytogenetically normal mentally retarded males studied with two fragile-X DNA probes

Table 3

Subject	Age (years)	Race	(Z-score)				Discriminant value	Checklist score
			Testicular volume (ml)	Ear width (cm)	Bizygomatic diameter (cm)	Head breadth (cm)		
AB	7	B	3 (10.4)	3.9 (2.0)	11.0 (-2.7)	13.3 (-2.0)	1.8	8
KW	22	B	40 (4.9)	4.1 (2.8)	13.9 (0.0)	14.7 (-0.8)	0.2	18
EO	26	W	27 (2.2)	3.9 (1.3)	13.8 (-0.3)	15.2 (-0.2)	0.3	11
TB	29	W	20 (0.9)	4.4 (3.7)	13.0 (-1.7)	14.2 (-1.8)	0.6	10
SC	30	W	20 (0.9)	4.0 (1.8)	13.1 (-1.2)	14.9 (-0.7)	0.0	15
MN	33	B	28 (2.4)	4.2 (2.2)	13.1 (-1.4)	15.0 (-0.2)	1.1	6
DB	33	W	50 (6.8)	4.3 (3.2)	13.0 (-1.7)	14.3 (-1.7)	1.2	8
GR	35	W	37 (4.3)	4.0 (1.8)	12.8 (-2.0)	14.0 (-2.2)	0.3	17
CP	39	W	28 (2.4)	4.5 (4.2)	14.0 (0.0)	14.7 (-1.0)	0.2	12
JM	39	W	53 (7.5)	4.0 (1.8)	13.0 (-1.7)	14.2 (-1.8)	1.1	16
TM	41	B	78 (12.4)	4.2 (3.2)	13.6 (-0.5)	14.7 (-1.0)	2.3	7
DM	43	W	20 (0.9)	4.2 (2.8)	14.0 (0.0)	16.0 (1.2)	0.5	12
WS	44	B	20 (0.9)	5.0 (6.2)	13.6 (-0.5)	14.3 (-1.5)	1.1	14
DMC	46	W	32 (3.3)	4.2 (2.8)	14.7 (1.2)	16.2 (1.5)	0.7	14
TL	51	W	58 (8.4)	4.2 (2.8)	13.1 (-1.5)	14.6 (-1.2)	1.6	12
WH	53	W	26 (2.1)	4.3 (3.2)	13.7 (-0.5)	14.9 (-0.7)	0.5	11
ED	56	B	22 (1.3)	4.2 (3.2)	13.4 (-0.9)	13.4 (-2.9)	0.6	10
LM	56	W	50 (6.9)	4.4 (3.7)	13.8 (-0.3)	14.5 (-1.3)	0.8	12
CM	57	W	20 (0.9)	4.2 (2.8)	12 (-3.3)	14.2 (-1.8)	1.1	14
GF	63	B	20 (0.9)	3.8 (1.6)	11.8 (-3.6)	14.8 (-0.6)	2.0	2
<i>Summary (n = 20)</i>			$\bar{X} \pm SD$ 34±16.5; range 3-78	$\bar{X} \pm SD$ 4.3±0.3; range 3.8-5.0	$\bar{X} \pm SD$ 12.3±0.7; range 11.0±14.7	$\bar{X} \pm SD$ 14.6±0.7; range 13.3±16.2	$\bar{X} \pm SD$ 0.9±0.6; range 0-2.0	$\bar{X} \pm SD$ 11.4±3.9; range 2-18

Individual checklist data from cytogenetically normal mentally retarded males studied with two fragile-X DNA probes

Table 4

Subject	Age (years)	Race	MR	LE	LT	PC	FH of MR	HA	TD	SAS	HE	PS	HF	PBE	SC	HB	PEC	Checklist scores
AB	7	B	2	0	0	2	2	0	0	0	2	0	0	0	0	0	0	8
KW	22	B	2	2	2	0	2	2	0	0	2	0	2	0	1	2	1	18
EO	26	W	2	2	1	0	2	0	2	0	0	?	0	0	0	2	0	11
TB	29	W	2	2	0	0	2	0	0	0	0	?	2	0	0	0	2	10
SC	30	W	2	1	0	0	0	2	2	2	2	?	0	0	0	2	2	15
MN	33	B	2	1	1	0	2	0	0	0	0	?	0	0	0	0	0	6
DB	33	W	2	2	2	0	0	0	0	0	0	0	2	0	0	0	0	8
GR	35	W	2	2	2	0	?	0	2	2	2	?	1	0	2	2	0	17
CP	39	W	2	2	1	0	0	1	0	1	2	?	0	0	2	1	?	12
JM	39	W	2	0	2	2	0	2	0	2	2	0	2	0	0	2	0	16
TM	41	B	2	2	1	0	0	1	0	0	1	?	0	0	0	0	?	7
DM	43	W	2	2	0	?	0	2	1	2	0	1	0	0	0	0	2	12
WS	44	B	2	2	0	0	0	0	2	2	2	?	2	0	0	0	2	14
DMC	46	W	2	0	2	0	0	2	2	2	0	?	0	0	0	2	2	14
TL	51	W	2	2	2	0	?	2	0	2	0	?	0	0	0	0	2	12
WH	53	W	2	2	1	0	2	2	0	0	0	?	2	0	0	0	?	11
ED	56	B	2	0	0	0	2	2	2	0	0	2	0	0	0	0	0	10
LM	56	W	2	2	2	0	?	2	0	2	0	0	2	0	0	0	0	12
CM	57	W	2	2	0	0	0	1	2	2	2	0	1	0	0	2	0	14
GF	63	B	2	0	0	0	?	0	0	0	0	0	0	0	0	0	0	2
Summary: (n = 20)																		
	$\bar{X} \pm SD$	13W:7B	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
	40.2 $\pm$ 13.8	2 $\pm$ 0	1,460.9	1.0 $\pm$ 0.9	0.2 $\pm$ 0.6	0.8 $\pm$ 0.9	1.0 $\pm$ 0.9	1.0 $\pm$ 1.0	0.8 $\pm$ 1.0	1.0 $\pm$ 1.0	0.8 $\pm$ 1.0	0.3 $\pm$ 0.7	0.8 $\pm$ 1.0	0 $\pm$ 0	0.2 $\pm$ 0.6	0.8 $\pm$ 1.0	0.8 $\pm$ 1.0	11.4 $\pm$ 3.9;
	range																	range
	7-63																	2-18