Correlation of skeletal muscle biopsy with phenotype in the familial macrocephaly syndromes

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Abstract

The muscle biopsy results from 14 children with macrocephaly and hypotonia/weakness were correlated with clinical findings compatible with any of the autosomal dominant macrocephaly syndromes. Thirteen of the 14 had evidence of lipid storage myopathy, either generalised or focal. All 13 had examinations consistent with either benign familial macrocephaly, Ruvalcaba-Myhre-Smith syndrome, or Bannayan-Zonana syndrome. These results suggest that all three of these disorders may represent phenotypic variability at a single genetic locus.

Considerable controversy and confusion surround the nomenclature and genetic basis of the familial macrocephaly-hamartoma syndromes, particularly the Ruvalcaba-Myhre-Smith (RMS),¹ Riley-Smith (RS),² and Bannayan-Zonana (BZ)³ phenotypes.^{4,5} Published information consists predominantly of case reports or small series, except for a few older reports which generally contain little or no data applicable to resolving this issue. Macrocephaly with an autosomal dominant pattern of inheritance appears to be the most important unifying feature among the clinical findings reported for these disorders, but hamartomatous lesions are also quite common. Hypotonia associated with gross motor or global developmental delay appears to be prevalent and has in some cases of RMS been correlated with a characteristic myopathy.⁶ At present this lipid storage myopathy (LSM), described in several children and adults with the RMS phenotype, is probably the most specific marker for any of these conditions. Accordingly, a retrospective study was undertaken to assess the prevalence of LSM in children with macrocephaly and hypotonia/weakness in order to determine its prevalence in phenotypes other than RMS as a way of shedding additional light on the aetiology of these disorders.

Methods

Fourteen children, 11 boys and three girls, ranging in age from 2 weeks to 9 years, who had been referred for the evaluation of hypotonia or weakness, were found to have macrocephaly based on occipitofrontal head circumferences greater than or equal to 2 SD above the mean for age. As part of routine neuromuscular evaluation, percutaneous muscle biopsies

were obtained in 13 children from the quadriceps femoris or vastus lateralis muscles or both and processed using techniques described previously.⁷ In one child an open biopsy was performed and processed using substantially similar methods. As part of the usual array of histochemical analyses the amount of intracellular neutral lipid, which is found predominantly in type 1 fibres, was qualitatively estimated by a neuropathologist unaware of the clinical diagnoses. Thirteen of the biopsies were examined by one pathologist and the most recent sample by a second at a different institution. Each sample was sent with either the patient's name and hospital number alone, or with the addition of the clinical diagnosis 'rule out myopathy'. The children and, in all but one case, their parents had physical examinations and multigeneration family histories were obtained. One child was adopted and no information was available regarding his parents.

An attempt was made to categorise each child or family into a single phenotype according to the clinical features. Since well defined criteria have not been agreed upon and considerable overlap among reported cases of these disorders has been observed, this was a somewhat subjective undertaking. Children and families with macrocephaly and hypotonia/weakness alone were considered to have BFM. This same constellation of features with the addition of lipomas, penile macules, or intestinal polyps was considered to be RMS. Where macrocephaly, hypotonia, and lipomas coexisted the BZ phenotype was used. A young child with macrocephaly and hypotonia/weakness alone, who presumably had not yet had sufficient time to manifest any of the other abnormalities, was considered to have the same phenotype as the affected parent and other family members.

Results

Four of the 14 children had soft tissue masses, apparently lipomatous lesions based on clinical evaluation. All four were boys and three had penile macules. Macrocephaly was identified in one parent of 12 of the 14 children (12 of 13 if the adopted child is excluded from this analysis). In addition, when other family members were available for head circumference measurements, a pedigree consistent with the expected autosomal dominant pattern of inheritance was found. Three (two male, one female) of the 12 macrocephalic parents had

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Received 27 February 1991.
Revised version accepted 2 May 1991.

Table 1 Patient characteristics and biopsy results.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age	6 y	15 mth	3 y	14 mth	3 y	3 y	5 mth	3 y	9 y	4 y	4 y	8 y	3 mth	2 w
Sex	M	F	F	F	M	M	M	M	M	M	M	M	M	M
Hamartomata	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parental macrocephaly	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parental (familial) hamartomata	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum carnitine	N	+	+	N	+	+	+	N	+	N	+	+	+	+
Biopsy: lipid	+	+	+	+	+	+	+	F	+	+	+	+	+	+
Biopsy: fibre size variation	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenotype	RMS	BFM	BFM	BFM	RMS	BZ	BFM	BFM	RMS	BFM	BFM	?	MS	BZ

soft tissue masses, again appearing to be lipomatous, and both men had penile macules.

All 12 children with familial macrocephaly had increases in both the size and number of neutral lipid droplets, as assessed by oil red O histochemistry, in type 1 skeletal muscle fibres, as did the adopted child (table 1). In two of these 13 the lipid increase was focal. Ten of the 13 children with increased lipid also had fibre size variation, generally with type 1 fibres larger than expected and type 2 fibres smaller. This fibre size variation was initially determined subjectively by the neuropathologist, but subsequent measurements of the type 1 and 2 fibres supported these observations when compared with age related standards. The single child with non-familial macrocephaly had a normal appearing biopsy in spite of an abnormal EMG.

Discussion

Saul and Stevenson,⁸ Dvir *et al.*,⁹ and Cohen⁴ have suggested previously that the syndromes described by Bannayan, Ruvalcaba, and Riley are the same disorder but differ phenotypically because of variable expression, but these authors do not appear to be suggesting that autosomal dominant (benign familial) macrocephaly (BFM) should also be included in this grouping. Only anecdotal data were offered in support of this conjecture, however. Cohen,⁴ in particular, has noted "lumping in non-biochemically defined disorders appears to be more a matter of clinical judgement than of 'scientific proof'." The present analysis was undertaken in an effort to clarify this situation using a fairly specific histochemical marker, LSM. Although muscle biopsies showing LSM may be seen in a variety of clinical situations, particularly primary and secondary disorders of carnitine metabolism, the reported frequency of this abnormality in children has generally been quite low.¹⁰ There is no reason to believe that hypotonia alone is associated with LSM. A large percentage of the nearly 300 muscle biopsies reviewed for this study were from children referred for the evaluation of hypotonia. The only hypotonic children with LSM had either associated macrocephaly, as noted below, or clear evidence of a specific metabolic disorder. It is therefore unlikely that LSM would be found associated coincidentally with macrocephaly. The other observed biopsy abnormality, fibre size variation, although not uncommon in hypotonic children, appears to be somewhat different in the reported patients with the RMS phenotype, since in addition to the small type 2 fibres

the type 1 fibres appeared to be larger than expected, an apparently unusual feature.

Evidence of LSM was found in all of the children with familial macrocephaly. In some cases the intracellular lipid was increased only in localised areas of the biopsy sections, but the neuropathologist noted this in reports written before discussion of the clinical information, making it unlikely that in these less well defined and perhaps somewhat subjective interpretations any bias based on clinical information was introduced. As an additional investigation of possible observer bias, all biopsy records of children with evidence of LSM were reviewed. Excluding the children known to have macrocephaly, in all but two other cases specific aetiologies for LSM, generally genetic or acquired metabolic disorders, had been identified. Further investigation of these two cases showed that both children had macrocephaly with a family pedigree containing a minimum of one affected parent. They have therefore been included in this analysis. Since a specific aetiology was identified in 100% of the children not in this study with biopsy evidence of LSM, our approach to identifying lipid storage myopathy would appear to be fairly objective. The biopsy results were consistent across all of the macrocephaly phenotypes observed. Biopsies from children with autosomal dominant macrocephaly alone were indistinguishable from those in which the clinical features were consistent with the BZ and RMS phenotypes, both with regard to the lipid distribution and the fibre size variation. Similarly, no obvious differences could be discerned between biopsies from boys or girls, although with only three girls included the numbers are too small to allow any great degree of confidence in this observation (table 2). This high male/female ratio is puzzling for a condition with a well defined autosomal dominant mode of inheritance. Although it is probably remotely possible that boys are more likely to be referred for evaluation of hypotonia or weakness because of higher societal

Table 2 Summary of correlations between biopsy results and clinical phenotype.

Phenotype	Biopsy results		
	GLSM	FLSM	FSV
BFM	5/7	1/7	5/7
BZ	1/2	1/2	2/2
RMS	3/3	0/3	2/3
MS	0/1	0/1	0/1
Uncertain	1/1	0/1	1/1
Totals	10/14	2/14	10/14

GLSM = generalised lipid storage myopathy.

FLSM = focal lipid storage myopathy.

FSV = fibre size variation.

expectations for physically oriented behaviour, this seems unlikely as an explanation for the entire sex ratio anomaly.

None of the affected parents had weakness or hypotonia at the time their children were evaluated. In virtually every case where a reliable developmental history was available for the parent, indications of delayed motor milestones were evident. A muscle biopsy was obtained from one parent during an elective procedure to remove a large lipoma. The histochemical appearance of this sample was indistinguishable from the biopsies in the children reported here and also in previously reported adults with the RMS phenotype.⁶

The only child in this study without a macrocephalic parent had no abnormalities evident on his muscle biopsy despite the fact that he was profoundly hypotonic and had an abnormal EMG. Initially, with the exception of macrocephaly, he had an otherwise normal physical examination. As he grew older, however, he changed substantially and developed accelerated linear growth, particularly of the hands and feet. Ectopia lentis and echocardiographic features compatible with Marfan syndrome (MS) were subsequently detected. No evidence of homocystinuria was found after metabolic testing. Despite careful physical, ophthalmological, and echocardiographic examinations, neither parent had any evidence of MS. Neuromuscular problems do not seem to have been reported previously in infants with MS. Although in the absence of a family history the diagnosis of MS may not be absolutely certain, the physical, ophthalmological, and echocardiographic features in this child are highly suggestive of this disorder and fibrillin studies were also consistent.

The aetiology of the LSM in these children is unclear. Serum carnitine levels were normal in all four who were tested. One early report found normal muscle and serum carnitine levels in a single patient,⁶ but subsequent results suggest that the carnitine level may actually be low in skeletal muscle.¹¹ This finding could certainly explain the LSM and weakness but must be confirmed. In spite of extensive metabolic evaluations of the children in the present report, no evidence of any inborn error of metabolism which might produce a secondary carnitine deficiency was identified. In particular, urinary organic acids and acyl carnitine fractionation by fast atom bombardment of urine samples obtained after carnitine loading were normal. Reduced skeletal muscle carnitine levels, if confirmed, in the setting of normal serum levels could perhaps result from a membrane transport defect in skeletal muscle. The normal metabolic studies and the autosomal dominant inheritance pattern make an enzymatic defect unlikely.

Based on an informal review of nearly 300 of the author's patients, whose evaluations included muscle biopsies, LSM associated with macrocephaly was the most frequent single diagnosis, exceeding the incidence of relatively common childhood neuromuscular disorders, such as Duchenne muscular dystrophy and spinal muscular atrophy, by a considerable

amount. Since mildly affected children are probably not referred for evaluation if they do not fall too far behind in their gross motor development, the actual incidence of this condition may be even higher and it may easily be the most common myopathy of childhood. It must be remembered, however, that a biopsy from one of the previously reported patients was normal from one muscle group and markedly abnormal in another. This observation, coupled with the focal nature of the lipid anomaly in the biopsies in this report, makes it likely that not all children can be expected to have abnormal biopsy results and LSM should not be used as a necessary condition for the diagnosis of this condition.

Despite the fact that all of the children in this report were referred for evaluation of hypotonia or weakness rather than macrocephaly and hamartomata, this mode of ascertainment would not appear to introduce any systematic bias into the study which would alter the conclusions. Although skeletal muscle biopsies have not been obtained from children with familial macrocephaly and normal muscle strength and tone, previously published biopsy results in adults with the RMS phenotype but no evidence of weakness have also shown LSM,⁶ making it unlikely that children would be unaffected.

The presence of an unusual histochemical marker, LSM, in all evaluated children with the BZ, RMS, and BFM phenotypes would appear to be strong evidence in favour of unifying these previously separate disorders into a single autosomal dominant syndrome with macrocephaly, hamartomata, and LSM as the cardinal manifestations. Although many other associated abnormalities have been observed, none appears to be as characteristic as these three. Unfortunately, no children with the RS phenotype were identified in this study. One child with familial macrocephaly, haemangiomas, profound hypotonia, and normal social and cognitive development was evaluated, but the family declined a muscle biopsy. The findings on the neuromuscular examination were comparable to the children who did have biopsies, however, suggesting that similar histochemical results may also have been found.

The results of this study, although not completely ruling out the possibility of multiple non-allelic disorders with similar phenotypes, support unification of at least three macrocephaly phenotypes into a single disorder. They do not appear to be helpful with regard to reaching any conclusion about the issue of whether the observed phenotypic variability is a result of multiple alleles at a single locus or variable expression of a single allele, however. This question could be answered by more extensive pedigree analysis or molecular studies. In the author's experience a tendency toward pedigrees consistent for the presence or absence of hamartomata appears to be evident, but no formal statistical analysis has been undertaken. Conversely, within a single pedigree manifesting macrocephaly and hamartomata, considerable variability may be

observed, although to some extent this may reflect the age at which the patients are examined and other factors. For example, in at least one BZ family (family Or) reported by Miles *et al.*,¹² before the full delineation of the RMS phenotype penile macules were identified in several affected family members.

Histochemical examination of skeletal muscle biopsies for the discrete marker, LSM, appears to be useful for resolving clinical ambiguities related to the autosomal dominant macrocephaly syndromes and should help to further analysis and understanding of this disorder, particularly as molecular approaches are applied in the future.

This paper was presented in part at the American Pediatric Society/Society for Paediatric Research meeting, New Orleans, Louisiana, 29 April to 2 May 1991. The author wishes to acknowledge the assistance of the late Dr David Hollister whose laboratory performed the fibrillin studies on the patient with Marfan syndrome.

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