Genetics inMedicine | BRIEF REPORT

Macrocephaly associated with the *DICER1* syndrome

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Purpose: Germ-line mutations in *DICER1* increase the risk of various tumors, including pleuropulmonary blastoma. Macrocephaly and symmetric overgrowth have been reported in some, but not all, patients with mosaic *DICER1* RNase IIIb mutations. The prevalence of these features in individuals with constitutional germ-line *DICER1* mutations is unknown.

Methods: We analyzed prospectively collected auxology data from 67 *DICER1* mutation carriers and 43 family controls. We assessed differences between groups using an exact test for proportions and generalized estimating equations for continuous dependent variables.

Results: Twenty-eight *DICER1* mutation carriers (42%) were macrocephalic, and none had an occipitofrontal circumference (OFC) below the third centile, which significantly differed from family controls, of whom five were macrocephalic (12%) and two had OFC below the third centile (5%) ($P < 0.001$). *DICER1* mutation carriers were taller than familial controls after controlling for gender ($P = 0.048$), but similar proportions of both groups were above the 97th centile of population norms. Head circumference remained increased after adjusting for differences in height.

Conclusion: For the first time, we establish macrocephaly as a common finding in the *DICER1* syndrome. Like some other tumor-predisposition disorders, macrocephaly may be a useful, albeit a subtle, clinical clue to the *DICER1* syndrome diagnosis.

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INTRODUCTION

Pleuropulmonary blastoma (PPB), an embryonal sarcoma and the most common pediatric primary lung malignancy,[1] is the hallmark tumor of the *DICER1* syndrome.[2] Somatic second hits in one of five "hotspot" amino acids of the *DICER1* RNase IIIb domain are necessary for malignant transformation of PPB and other *DICER1*-associated tumors.[3,4] Germ-line *DICER1* mutations are also associated with a variety of other neoplasms, including cystic nephroma, nasal chondromesenchymal hamartoma, ciliary body medulloepithelioma, pituitary blastoma, as well as Sertoli-Leydig cell tumor and other ovarian sex cord stromal tumors.[5]

PPB is a serious complication of the *DICER1* syndrome. PPB progresses through stages of malignant transformation, from type I (cystic) to type II (partially cystic/solid) and type III (solid). The 5-year overall survival for later-stage patients is 71% (type II) and 53% (type III). Relapse and metastasis, primarily to the central nervous system, is responsible for much of the mortality.[6,7] Fortunately, early detection and subsequent surgical resection of PPB can be curative.[8] However, this is feasible only if *DICER1* mutation carriers are identified and screened (by chest computed tomography) as early in life as possible.

The presence of easily detected, nonmalignant phenotypic features can prompt alert clinicians to consider the diagnosis of an occult tumor-predisposition disorder in an otherwise healthy child. For example, the distinctive skin findings and increased head circumference in neurofibromatosis type 1 and Cowden syndrome may appear before any syndrome-associated neoplasia. In the *DICER1* syndrome, lung cysts, cystic nephroma, or family history of multinodular goiter have been used to identify *DICER1* mutation carriers (hereafter, "*DICER1* carriers") before the development of malignancy.[8,9] Because the *DICER1* syndrome was recognized relatively recently, systematic evaluations of growth have not been reported, although there have been accounts of developmental delay, macrocephaly, and overgrowth in patients with mosaic *DICER1* "hotspot" RNase IIIb mutations.[10] Other accounts have not reported overgrowth or developmental delay in these patients.[11] In our natural history study of the *DICER1* syndrome, we comprehensively evaluated individuals with germ-line *DICER1* mutations and family controls. We analyzed auxology data from our cohort, particularly head circumference and height measurements, to characterize previously unrecognized *DICER1*-associated disease features that may be useful in identifying individuals and families at risk of PPB.

MATERIALS AND METHODS

Study participants

The National Cancer Institute protocol "*DICER1*-Related Pleuropulmonary Blastoma Cancer Predisposition Syndrome: A Natural

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History Study" (National Cancer Institute protocol 11-C-0034; NCT 01257597) is open to individuals with *DICER1*-associated tumors and their family members. Between November 2011 and December 2014, 134 participants from 31 families were evaluated at the National Institutes of Health Clinical Center. CLIA-certified germ-line *DICER1* mutation testing was conducted at Ambry Genetics (Aliso Viejo, CA) and the Children's National Medical Center (Washington, DC). Five children without detectable germ-line *DICER1* mutations but who harbored *DICER1*-associated tumors were considered separately. We compared individuals carrying pathogenic germ-line *DICER1* mutations ($n = 76$) with unaffected family members lacking pathogenic *DICER1* mutations ($n = 53$). The study was approved by the National Cancer Institute's institutional review board, and all participants, or their parents or guardians, provided written, informed consent.

Dysmorphology and reference curves

Height was measured by clinical staff at the National Institutes of Health Clinical Center using stadiometers. Head occipitofrontal circumference (OFC), arm span, and lower-segment length were recorded using measuring tapes, as described by Gripp et al.[12] Upper-segment length was calculated by subtracting the lower-segment length (distance from the top of the pubic symphysis to the floor) from the height. The ratio of upper-segment length to lower-segment length (US/LS) was then calculated. We compared observed measurements with age- and gender-appropriate reference charts for height[13] and OFC.[14] OFC and height are strongly correlated, and we used OFC-for-height references for those aged 18 years and older.[15] We considered abnormal height to be below the 3rd centile or above the 97th centile and macrocephaly as greater than the 97th centile in published reference populations.

Statistical analyses

We tested differences in proportions using either a chi-squared test or an exact test when frequencies were low ($n < 5$). We assessed differences in continuous descriptive characteristics using the Wilcoxon rank-sum test. We fit generalized estimating equations to the continuous measurements of OFC and height in adults to account for correlation within families, and we used robust standard errors. All tests were two-sided; we considered $P < 0.05$ significant, and analyses were performed using Stata/SE version 13.1 (Stata, College Station, TX).

RESULTS

Individuals ($n = 19$) were excluded from our analyses because of missing data on any of the following: OFC ($n = 14$), US/LS ratio ($n = 11$), and arm span ($n = 9$). The remaining 110 participants comprised the analytic data set. Cohort demographics are described in **Supplementary Table S1** online.

Macrocephaly was more frequent in *DICER1* carriers than in family controls

DICER1 carriers differed from family controls in the distribution of head circumference (**Figure 1a**): 28 *DICER1* carriers (42%) were macrocephalic, and none had an OFC below the third centile, versus family controls, of whom five were macrocephalic (12%) and two had an OFC below the third centile (5%) ($P < 0.001$). This difference between *DICER1* carriers and controls remained significant after stratification by gender. Seventeen females with a *DICER1* mutation (50%) were macrocephalic, and none had an OFC below the third centile, versus three controls with macrocephaly (20%) and two with OFC below the third centile (13%) ($P = 0.024$). Similarly, 11 males with a *DICER1* mutation (33%) were macrocephalic compared with only two controls (7%) ($P = 0.026$). When plotted against the reference curves published by Rollins et al.,[14] OFC was systematically increased in *DICER1* carriers compared with both population norms and family controls (**Figure 1b,c**). Among those aged ≥18 years, *DICER1* carriers had a 2.25-cm increase in OFC (95% confidence interval: 1.2–3.3; $P < 0.001$), after adjusting for gender. We did not estimate the magnitude of the increase in children because the small number of pediatric controls precluded modeling of the nonlinear relationship between age and OFC. However, 8 *DICER1* carriers aged <18 years (25%) were macrocephalic compared with one control aged <18 years (10%), though the difference was not statistically significant ($P = 0.219$). The five children with a *DICER1*-associated tumor but no detectable *DICER1* germline mutation had a distribution of OFC similar to that of family controls (**Figure 1b,c**). OFC did not correlate with *DICER1* mutation location or type (**Supplementary Figure S1** online and **Supplementary Table S2** online).

There were no differences in height between *DICER1* carriers and controls

Although *DICER1* mutation carriers were taller than familial controls after controlling for gender ($P = 0.048$), the proportion of individuals with height >97th general population centile was similar between *DICER1* carriers (12%) and controls (7%) ($P = 0.52$) (data not shown). Tall stature was not more prevalent in either females ($P = 0.414$) or males ($P = 1.0$) with the *DICER1* syndrome. The *DICER1* syndrome in adults was not associated with greater height after adjusting for gender (difference = 2.5 cm; 95% confidence interval: −1.0–6.1; $P = 0.160$).

Larger head circumference in *DICER1* carriers was independent from differences in height

As noted above, OFC and height are strongly correlated among the general population. Using the reference curves from Bushby et al.[15] that adjust for height among those aged ≥16 years, the distribution of OFC for height among adults (age ≥18 years) with *DICER1* differed significantly from that of family controls (**Figure 2a**). Ten adults with the *DICER1* syndrome (29%) had an OFC above the 97th centile and none had an OFC below the 3rd centile, versus two controls with an OFC above the 97th centile (6%) and two controls with an OFC below the 3rd centile (6%) ($P = 0.017$). Stratifying by gender did not detect significant differences between groups. Eight women with the *DICER1* syndrome (33%) had an OFC above the 97th centile

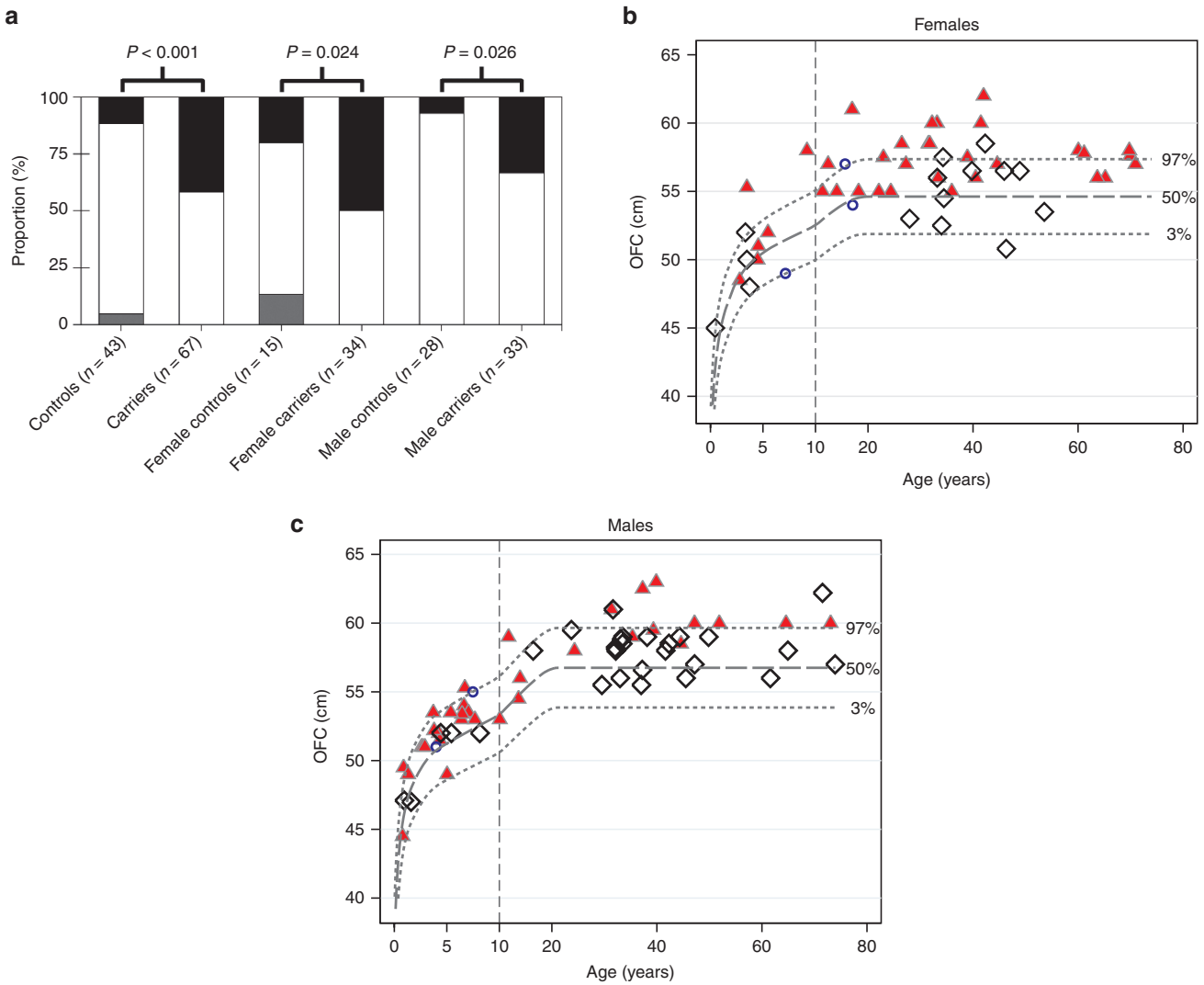

Figure 1 Occipitofrontal circumference (OFC)–for–age in the *DICER1* syndrome. (**a**) Abnormal OFC for age. Proportions between the 3rd and 97th centiles (white), below the 3rd centile (gray), or above the 97th centile (black) in *DICER1* mutation carriers and family controls. *P* values are for the Fisher exact test of differences between groups. (**b**) Females. Red triangles indicate *DICER1* mutation carriers. White diamonds represent family controls. Blue circles represent girls without a detectable germ-line *DICER1* mutation but who harbor a *DICER1*-associated tumor (7-year-old: type II pleuropulmonary blastoma (PPB); 15.5-year-old: Sertoli-Leydig cell tumor; 17-year-old: type II PPB). The dashed lines indicate the 97th, 50th, and 3rd centiles of OFC for age reported in Rollins et al.[14] The vertical dashed line at age 10 years indicates a change in the scale of the x axis to allow for better resolution of children's values. (**c**) Males. Blue circles represent boys without a detectable germ-line *DICER1* mutation but who harbor at least one *DICER1*-associated tumor (4-year-old: type I PPB and cystic nephroma; 7.7-year-old: type II PPB only).

and none had an OFC below the 3rd centile compared with one woman control (9%) with an OFC above the 97th centile and two (18%) with an OFC below the 3rd centile ($P = 0.065$). Two males with the *DICER1* syndrome had an OFC above the 97th centile (18%) compared with one male control (5%) ($P = 0.25$). Qualitatively, OFC-for-height in *DICER1* carriers was larger than expected (Figure 2b,c). Among adults, the *DICER1* syndrome was associated with an average increase in OFC of 1.92 cm (95% confidence interval: 1.1–2.8; $P < 0.001$) after adjusting for gender and height.

Arm span–to–height ratio and long-bone growth were proportional in patients with the *DICER1* syndrome

No significant differences in the US/LS or arm span–to–height ratios were observed. The US/LS ratio in *DICER1* carriers (mean = 0.977; SD = 0.087) was similar to that in family controls (mean = 0.950; SD = 0.087) ($P = 0.443$) among those aged 18 years and older. The relationship between arm span and height were linear, as is expected in the general population, and no significant differences were observed between *DICER1* carriers (mean = 1.022; SD = 0.043) and controls (mean = 1.024; SD

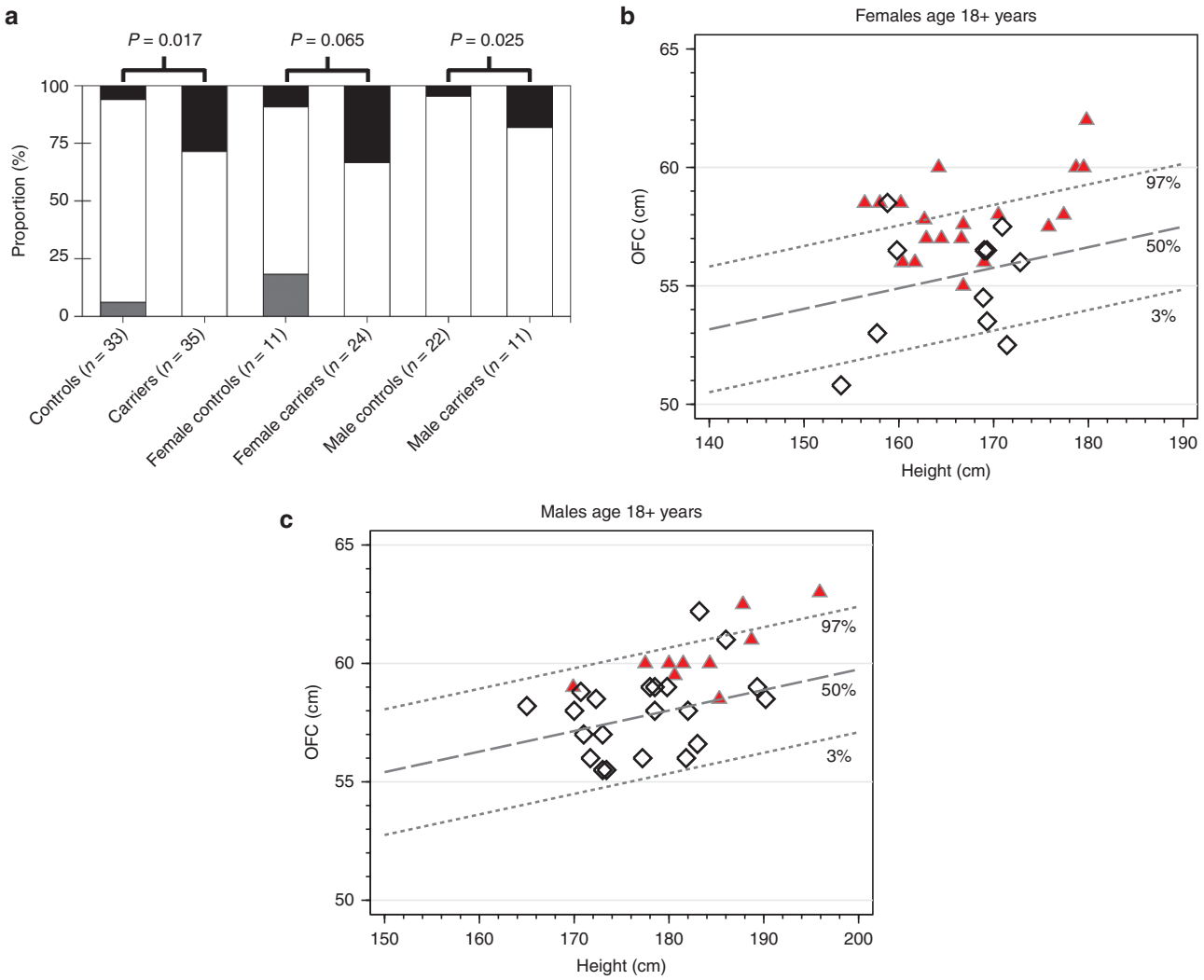

Figure 2 Occipitofrontal circumference (OFC)–for–height in the *DICER1* syndrome (mutation carriers and controls ≥18 years old). (**a**) Abnormal head circumference for height. Proportions between the 3rd and 97th centiles (white), below the 3rd centile (gray), or above the 97th centile (black) in *DICER1* mutation carriers and family controls. *P* values are for the Fisher exact test of differences between groups. (**b**) Females. Red triangles indicate *DICER1* mutation carriers. White diamonds represent family controls. The dashed lines indicate the 97th, 50th, and 3rd centiles of OFC for height reported by Bushby et al.[15]. (**c**) Males.

= 0.028) (P = 0.747). Adjustment for neither gender nor age meaningfully affected the results (data not shown).

DISCUSSION

In our study macrocephaly was observed in 42% of *DICER1* carriers evaluated at the National Institutes of Health Clinical Center. Other growth measurements were normal relative to the general population, that is, *DICER1* carriers were not abnormally tall, but adults with *DICER1* were taller on average compared with family controls. After adjusting for these differences in height, the association between macrocephaly and *DICER1* mutation status persisted. Measurements of long-bone growth (arm span–to–height and US/LS ratios) were within normal ranges.

In mice *Dicer1* is a haploinsufficient tumor-suppressor gene[16]; our data show for the first time that human macrocephaly is a phenotype significantly associated with *DICER1* haploinsufficiency. In the *DICER1* syndrome the macrocephaly is relatively (but not disproportionately) increased and is not associated with somatic overgrowth.[17] Klein et al.[10] reported macrocephaly and symmetric overgrowth in two children with mosaic missense "hotspot" mutations in the RNase IIIb domain of *DICER1*, along with developmental delay and Wilms tumor, in a constellation of findings they termed the "GLOW" (global developmental delay, lung cysts, overgrowth, and Wilms tumor) syndrome. The authors also identified 10 candidate dysregulated 3p microRNAs that target negative regulators of the mammalian target of rapamycin, transforming growth factor-β and mitogen-activated protein kinase signaling pathways, including *PTEN*, *TSC*, and *NF1*. They hypothesize that an imbalance in specific 3p microRNAs arising from *DICER1* RNase IIIb mutations lead to excessive cell and tissue growth

and tumor predisposition. Mosaic *DICER1* RNase IIIb domain mutations are associated with a more severe neoplastic phenotype.[11,18] Many of the GLOW phenotype features, including macrocephaly and overgrowth, were not observed in a set of four patients with mosaic *DICER1* RNase IIIb mutations.[11] The differences in these studies may be attributable to the pleiotropy and phenotypic variability inherent in mosaicism and highlight the need for a systematic, statistically grounded approach to syndrome delineation.

It is well known that haploinsufficiency of *PTEN* (Cowden and Bannayan-Riley-Ruvalcaba syndromes) and *NF1* (neurofibromatosis type 1) is associated with macrocephaly. It is interesting to note that these genes are also dysregulated in *DICER1* mosaicism.[10] Increased head circumference in neurofibromatosis type 1 is hypothesized to be a secondary skeletal manifestation of brain overgrowth,[19] presumably caused by dysregulation of key growth pathways. The often pronounced macrocephaly in these disorders can be a useful clinical clue to their diagnosis. The role of these genes as intermediaries of posited brain overgrowth with secondary skeletal growth in the *DICER1* syndrome phenotype merits further study.

Our analysis is limited by the biases inherent in using cross-sectional data to assess growth. Longitudinal analyses are needed to discern when OFC increases and would inform future studies of the underlying mechanism of this growth. Moreover, families enrolled in the study were accessioned because of a history of a *DICER1*-associated tumor. Ascertainment bias may have missed clinically asymptomatic *DICER1* carriers with milder phenotypes. Finally, measurements were made by multiple observers rather than a single physician. However, the measurements of OFC, arm span, and height are unlikely to vary substantially enough between observers to account for the large difference observed in *DICER1* carriers in this study.

In summary, our study is the first to document macrocephaly as a non-neoplastic feature of the *DICER1* syndrome. Further analyses of longitudinal data may shed light on the developmental processes underlying the macrocephaly and point to the role of *DICER1* in auxology. Like other, better-known tumor-predisposition disorders, macrocephaly may be a useful, if subtle, clinical clue to the diagnoses of the *DICER1* syndrome.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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