Limb girdle muscular dystrophies: update on genetic diagnosis and therapeutic approaches
Vincenzo Nigro, Stefania Aurino and Giulio Piluso

Introduction
The term limb girdle muscular dystrophy (LGMD) broadly defines a progressive weakness that begins from the proximal limb muscles, due to a genetic defect that is distinct from the X-linked dystrophinopathy. The disease is not congenital, with the age at onset of symptoms varying from early childhood to late adulthood [1]. The progression of muscle wasting is usually symmetric, with a variability among individuals and genetic subtypes. Before considering the diagnosis of LGMD [2], other conditions need to be excluded, such as facioscapulohumeral muscular dystrophy, dystrophinopathies, myotonic dystrophy, and metabolic myopathies. The milder the symptoms are, the more difficult is the diagnosis. MRI may be helpful to characterize the severity and pattern of muscle involvement [3].

Muscle biopsy shows a diffuse variation in fiber size, necrosis, regeneration, and fibrosis, but the degree of these factors is variable and does not parallel the clinical severity. On the basis of the histological features alone, there is little, if any, possibility of diagnosing an LGMD or a specific LGMD form, but it is possible to discriminate LGMD from inflammatory myopathy, myofibrillar myopathy, or neurogenic atrophy.

Classification
The primary distinction is between the autosomal dominant (LGMD1, Table 1) and the autosomal recessive forms (LGMD2, Table 2), with a progressive alphabetical letter indicating the order of gene mapping [4]. There are, however, about one third of LGMD patients without any genetic classification. According to the disease mechanisms, the LGMDs may be grouped as follows: dystrophin–dystroglycan complex defects LGMD2CDEFIKMNOP; membrane defects LGMD1C, LGMD2BL; enzymatic LGMD2AH; sarcomeric LGMD1A, LGMD2G; and nuclear lamina LGMD1B.

LGMD1
Eight LGMD1 loci have so far been identified, but the heterogeneity is expected to be greater (Table 1). The LGMD1 forms have an adult-onset and are milder,
LGMD2A (calpain 3)

LGMD2A is caused by calpain 3 (CAPN3) mutations and represents 20–40% of cases. It is due to a high heterozygote frequency (1:100–120), carrying a large spectrum of different CAPN3 pathogenic changes [457]; some of them (17) for their frequency are included in the dbSNP database [16]. CAPN3 is a 94 kDa muscle-specific protein similar to ubiquitous calpain 1 and 2, contain specific mutation sequences (NS, IS1, and IS2). Upon stimulation, CAPN3 both activates and deactivates rapidly through autolysis of the insertion sequences [17]. In the sarcomeres, CAPN3 binds to connectin/titin and changes its localization from the M-lines to the N2A regions as the sarcomeres extend. The mobility of calpain 3 between the M-lines and the cytosol may have a key role in physical stress, and it is compromised in muscular dystrophy when its protease activity has been lost [18]. CAPN3 can cleave PIAS proteins and negatively regulates PIAS3 sumoylation activity [19].

CAPN3 mutations are associated with two main clinical features: LGMD with a recognizable clinical pattern of ‘calpainopathy’, characterized by arthropathy, scapular spondylolysis, and muscle weakness. The muscle biopsy shows nemaline bodies and central cores. The mutation spectrum of CAPN3 includes several different types of mutations, including nonsense, frameshift, and missense mutations. Some patients with mutations in CAPN3 have a clinical phenotype similar to LGMD1B, while others show a more severe phenotype with early onset and rapid progression. The diagnosis of LGMD2A is confirmed by genetic testing, which shows a high heterozygote frequency (1:100–120) and a large spectrum of different CAPN3 pathogenic changes.

Table 1: Autosomal dominant limb girdle muscular dystrophy

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Animal model</th>
<th>Typical onset</th>
<th>Progression</th>
<th>Cardiomyopathy</th>
<th>sCK</th>
<th>Allelic disorders (OMIM #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD1A</td>
<td>Myotilin</td>
<td>Myo-7</td>
<td>Adulthood</td>
<td>Slow</td>
<td>Not observed</td>
<td>3–4X</td>
<td>MRM (609020)</td>
</tr>
<tr>
<td>LGMD1B</td>
<td>Lamin A/C</td>
<td>Lamnm-1</td>
<td>Variable (4–38y)</td>
<td>Slow</td>
<td>Often observed</td>
<td>1–6X</td>
<td>Spheroid body myopathy (182920)</td>
</tr>
<tr>
<td>LGMD1C</td>
<td>Caveolin 3</td>
<td>Cas3-3</td>
<td>Childhood</td>
<td>Slow/moderate</td>
<td>Frequent</td>
<td>10X</td>
<td>EMD3 (181350)</td>
</tr>
</tbody>
</table>

LGMD1D 6q22 Adulthood Slow Not observed 1–3X
LGMD1E 7q38 Adulthood Slow Not observed 2–4X
LGMD1F 7q31.1 Variable (1–58y) Quite rapid Not observed 1–9X
LGMD1G 4p21 Adulthood Slow Not observed 1–10X
LGMD1H 3p23–p25 Variable (10–50y) Slow Not observed 1–10X

Table 1: Autosomal dominant limb girdle muscular dystrophy

<table>
<thead>
<tr>
<th>Disease</th>
<th>Locus</th>
<th>Name</th>
<th>Exons no</th>
<th>Protein</th>
<th>Animal model</th>
<th>Typical onset</th>
<th>Progression</th>
<th>Cardiomyopathy</th>
<th>sCK</th>
<th>Allelic disorders (OMIM #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD1A</td>
<td>5q31.2</td>
<td>TTID</td>
<td>10</td>
<td>Myotilin</td>
<td>Myo-7</td>
<td>Adulthood</td>
<td>Slow</td>
<td>Not observed</td>
<td>3–4X</td>
<td>MRM (609020)</td>
</tr>
<tr>
<td>LGMD1B</td>
<td>1p21</td>
<td>LMNA</td>
<td>12</td>
<td>Lamin A/C</td>
<td>Lamnm-1</td>
<td>Variable (4–38y)</td>
<td>Slow</td>
<td>Often observed</td>
<td>1–6X</td>
<td>Spheroid body myopathy (182920)</td>
</tr>
<tr>
<td>LGMD1C</td>
<td>3p25.3</td>
<td>CAV3</td>
<td>2</td>
<td>Caveolin 3</td>
<td>Cas3-3</td>
<td>Childhood</td>
<td>Slow/moderate</td>
<td>Frequent</td>
<td>10X</td>
<td>EMD3 (181350)</td>
</tr>
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LGMD1D 6q22 Adulthood Slow Not observed 1–3X
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a Indicates the age of onset of the majority of patients reported; early childhood has been defined as a period of life between 0–8 years of age; late childhood 9–12 years; adolescence 13–17 years; young adulthood, 18–35 years, etc.

b Also indicates mild signs of cardiac involvement.

c Indicates the range of serum creatine kinase (sCK) levels that is observed in about 80% of patients.

d Only indicates allelic disorders that have been included in the Online Mendelian Inheritance in Man (OMIM) with the indicated number.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Locus</th>
<th>Name</th>
<th>Exons no.</th>
<th>Gene</th>
<th>Protein</th>
<th>Animal model</th>
<th>Clinical phenotype</th>
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</thead>
<tbody>
<tr>
<td>LGMD2A</td>
<td>15q15</td>
<td>CAPN3</td>
<td>24</td>
<td>Calpain 3</td>
<td>CAPN3&lt;sup&gt;−/−&lt;/sup&gt;; CAPN3&lt;sup&gt;Cavca&lt;/sup&gt;</td>
<td>Adolescence</td>
<td>Moderate/rapid; Rarely observed</td>
</tr>
<tr>
<td>LGMD2B</td>
<td>2p13.2</td>
<td>DYSF</td>
<td>56</td>
<td>Dysferlin</td>
<td>SJL/J, Dysf&lt;sup&gt;Lm&lt;/sup&gt;</td>
<td>Young adulthood</td>
<td>Slow; Possible</td>
</tr>
<tr>
<td>LGMD2C</td>
<td>13q12</td>
<td>SGCG</td>
<td>8</td>
<td>Sarcoglycan</td>
<td>Sgcg&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Early childhood</td>
<td>Rapid; Often severe, rare in 2D</td>
</tr>
<tr>
<td>LGMD2D</td>
<td>17q21.33</td>
<td>SGCA</td>
<td>10</td>
<td>α-Sarcoglycan</td>
<td>Sgca&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Early childhood</td>
<td>Rapid; Often severe, rare in 2D</td>
</tr>
<tr>
<td>LGMD2E</td>
<td>4q12</td>
<td>SGCB</td>
<td>6</td>
<td>β-Sarcoglycan</td>
<td>Sgcβ&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Early childhood</td>
<td>Rapid; Often severe, rare in 2D</td>
</tr>
<tr>
<td>LGMD2F</td>
<td>5q33</td>
<td>SGCD</td>
<td>9</td>
<td>δ-Sarcoglycan</td>
<td>BIC14.8, Sgcd&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Early childhood</td>
<td>Rapid; Often severe, rare in 2D</td>
</tr>
<tr>
<td>LGMD2G</td>
<td>17q12</td>
<td>TCAP</td>
<td>2</td>
<td>Telethonin</td>
<td>Tcap KO</td>
<td>Adolescence</td>
<td>Slow; Yes</td>
</tr>
<tr>
<td>LGMD2H</td>
<td>9q33.1</td>
<td>TRIM2</td>
<td>2</td>
<td>Tripartite motif containing 32</td>
<td>Trim2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Adulthood</td>
<td>Slow; Not observed</td>
</tr>
<tr>
<td>LGMD2J</td>
<td>2q24.3</td>
<td>TTN</td>
<td>312</td>
<td>Titin</td>
<td>MdMOK&lt;sup&gt;−/−&lt;/sup&gt; Ti ME1&lt;sup&gt;x2mX&lt;/sup&gt;</td>
<td>Young adulthood</td>
<td>Severe; Not observed</td>
</tr>
<tr>
<td>LGMD2K</td>
<td>9q34.1</td>
<td>POMT1</td>
<td>20</td>
<td>Protein-O-mannosyltransferase 1</td>
<td>Pomt1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Childhood</td>
<td>Slow; Not observed</td>
</tr>
<tr>
<td>LGMD2L</td>
<td>11p13-p12</td>
<td>ANO5</td>
<td>22</td>
<td>Anoctamin 5</td>
<td>Ano5&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Variable (young to late adulthood)</td>
<td>Slow; Not observed</td>
</tr>
<tr>
<td>LGMD2M</td>
<td>9q31</td>
<td>FKTN</td>
<td>11</td>
<td>Fukutin</td>
<td>Fukutin null</td>
<td>Early childhood</td>
<td>Moderate; Sometimes</td>
</tr>
<tr>
<td>LGMD2N</td>
<td>14q24</td>
<td>POMT2</td>
<td>21</td>
<td>Protein-O-mannosyltransferase 2</td>
<td>Pomt2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Early childhood</td>
<td>Slow; Rare</td>
</tr>
<tr>
<td>LGMD2O</td>
<td>1p34.1</td>
<td>POMGnTI</td>
<td>22</td>
<td>Protein O-linked mannosyl beta1, 2-N-acetylglucosaminyltransferase</td>
<td>POMGnT1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Late childhood</td>
<td>Slow; Not observed</td>
</tr>
<tr>
<td>LGMD2P</td>
<td>3p21</td>
<td>DAG1</td>
<td>3</td>
<td>Dystroglycan</td>
<td>Dag1 null</td>
<td>Early childhood</td>
<td>Moderate; Not observed</td>
</tr>
</tbody>
</table>

* Indicates the age of onset of the majority of patients reported; early childhood has been defined as a period of life from 0–8 years of age; late childhood 9–12 years; adolescence 13–17 years; young adulthood, 18–35 years, etc.

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wimming, weakness of the hip adductors, involvement of the posterior thigh muscles, and joint contractures [20]; a spectrum of variable phenotypes, often misdiagnosed [13,21], ranging from a common asymptomatic hyperCKemia to inflammatory disorders of muscle with eosinophilic infiltrates [22] or Becker muscular dystrophy (BMD)-like phenotypes. Eosinophils can be found in LGMD2A [23], but also in LGMD2C [24].

Western blot analysis is currently the ‘gold standard’ to identify LGMD2A. Loss of all CAPN3 bands by 2C4 (exon 1) and 12A2 (exon 8) [25] antibodies is specific, but the sensitivity is incomplete, because some LGMD2A patients may retain normal amounts of nonfunctional protein [26]. CAPN3 may be reduced in amount in other LGMDs (e.g. LGMD2B [27] and 2) [28] as a secondary effect. By immunohistochemistry, the complete absence of the 2C4 signal is 100% specific for LGMD2A [29].

Mutation detection is usually carried out by DNA analysis of all exons, but some intronic splice mutations can be overlooked [30] and heterozygous deletions [31] missed. The sensitivity is much higher by adding mRNA testing [32]. In this case also there is a problem of specificity, because many nonsense mutations await experimental proof of pathogenicity.

**LGMD2B (dysferlin)**

LGMD2B is caused by mutations in the dysferlin (DYSF) gene that is the second form in order of frequency (about 15–25%) in many geographical areas [10,15,33], but not everywhere [11]. Dysferlin is a ubiquitous 230 kDa transmembrane protein involved in calcium-mediated sarclemma resealing [34]. Although muscle inflammation is widely recognized in dysferlinopathy and dysferlin is expressed in immune cells, the contribution of the immune system to the pathology remains obscure.

DYSF mutations are associated with heterogeneous clinical pictures ranging from severe functional disability to mild late-onset forms [35,36]. About 25% of cases are clinically misdiagnosed as having polymyositis [37]. The same mutations also cause Miyoshi myopathy (MM1) [38] and distal myopathy with anterior tibialis onset (DMAT1), but mixed phenotypes are possible. This classification into separate phenotypes does not reveal true disease differences [39].

Typical features of LGMD2B are: early adult onset; high serum creatine kinase (CK), higher than in LGMD2A [40]; prominent inflammatory infiltration; slow progression; and inability to stand on tiptoes, due to the weakness of the gastrocnemius and soleus. Fifty-three percent of the patients were very active and sporty before the onset of symptoms [41] and this suggests that a nonpenetration of DYSF mutations is possible. Regeneration seems to be attenuated [42].

Western blot analysis is very useful and specific, when less than 20% level of dysferlin has been identified [43*], although dysferlin can be also increased [35] or secondarily reduced [27]. Genetic testing is laborious for the huge number of exons to be screened and the lack of mutational hot spots. mRNA analysis is also reliable from monocytes, albeit with some splice differences [44].

**LGMD2C (gamma-sarcoglycan), LGMD2D (alpha-sarcoglycan), LGMD2E (beta-sarcoglycan), and LGMD2F (delta-sarcoglycan)**

Mutations in any of the four sarcoglycan genes (sarcoglycanopathies) constitute about 10–15% of all LGMDs [1,4,45], but 68% of the severe forms [46]. LGMD2D is the most prevalent form, but LGMD2C is common in the Maghreb and India [47] for the high allele frequency of 525delT and in gypsies for the C283Y allele [48]. The sarcoglycans are N-glycosylated transmembrane proteins that form a heterotetrameric complex linked to the dystrophin–dystroglycan complex [45].

The clinical picture of the sarcoglycanopathies is heterogeneous with both severe and mild forms that are also found in the same families [49]. In general, the disease is more severe and rapid than in the other LGMDs. The typical form has a childhood onset that resembles the intermediate forms of Duchenne/Becker muscular dystrophies usually with quadriiceps muscle wasting. Cardiomyopathy may occur in all forms [50,51], rarely in LGMD2D. In animal models, the delta sarcoglycan mutations are associated with cardiomyopathy [52,53]. Restrictive lung disease and hypventilation often require ventilatory assistance. Diagnosis may be made by Western blot or on section by immunofluorescence. LGMD2E and LGMD2F patients show most frequently the absence of the mutated and the secondary absence of nonmutated sarcoglycans, whereas LGMD2C patients may show the absence of gamma-sarcoglycan together with traces of the other nonmutated sarcoglycans. In LGMD2D cases, there is no rule. This could be explained by the presence of two other sarcoglycans (epsilon and zeta) that are nonmuscle homologues of alpha and gamma sarcoglycan. The genetic analysis is oriented to genotype common mutations or to sequences from DNA samples, the exonic regions of a specific sarcoglycan. More than in other LGMD genes, the sensitivity is lower without muscle mRNA testing and/or multiplex ligation-dependent probe amplification, because some nonobvious splice mutations are missed together with copy number mutations that are common in the gamma-sarcoglycan gene [54,55].
LGMD2G (Tcap/telethonin)
Mutations in titin cap (Tcap)/telethonin cause LGMD2G, one of the rarest forms of LGMD. Tcap provides links to the N-terminus of titin and other Z-disc proteins. Patients show adolescence-onset weakness initially affecting the proximal pelvic muscles and then the distal legs with calf hypertrophy. Recently, a patient with a homozygous nonsense mutation in the Tcap gene has been reported presenting with a congenital muscular dystrophy [56]. The Tcap gene has also been associated with cardiomyopathy [57], whereas common variants may play a role in genetic susceptibility to dilated cardiomyopathy [58]. Immunofluorescence and Western blot assays may show a telethonin deficiency. Full sequencing testing may be cost-effective in all cases, because the gene is only composed of two small exons.

LGMD2H (TRIM32)
Mutations in TRIM32 cause LGMD2H, a late-onset form that accounts for about 3% of LGMD. TRIM32 is a ubiquitous E3 ubiquitin ligase that belongs to a protein family comprising at least 70 human members sharing the tripartite motif (TRIM) [59]. The D487N mutation of TRIM32 was originally identified in the inbred population of Manitoba Hutterites [60] that may also show the more severe sarcotubular myopathy (STM) [61]. Other TRIM32 mutations were then identified in non-Hutterite LGMD2H patients [62,63]. Recently, two other LGMD2H patients have been described associated with STM morphotype [64]. In general, LGMD2H cannot be diagnosed without genetic studies. DNA sequencing of the unique coding exon is routinely performed, but in few laboratories. mRNA analysis is dispensable.

LGMD2I (FKRP), LGMD2K (POMT1), LGMD2M (fukutin), LGMD2N (POMT2), LGMD2O (POMGnT1), and LGMD2P (dystroglycan gene)
Mutations in these genes affect dystroglycan glycosylation and cause congenital muscular dystrophies, muscle–eye–brain disease or Walker–Warburg syndrome; however, some hypomorphic alleles are associated with LGMD [65,66**]. The most frequent LGMD gene in this group is FKRP that causes LGMD2I [67]. In some countries (England, Denmark, and Norway [68]), LGMD2I is more common than LGMD2A, for the high carrier frequency of the L276I allele (1 : 116), reported 377 times in the Leiden database. LGMD2I with both L276I alleles is generally milder than compound heterozygotes [69]. LGMD2I with the L276I allele is a muscular dystrophy that is clinically similar to BMD, with a late-childhood onset, calf hypertrophy, high serum CK, respiratory impairment, and cardiomyopathy that can also prevail [70,71]. A mild cognitive impairment of executive functions and visuo-spatial planning with aspecific MRI findings has been reported [72]. Myoglobinuria and myalgia following exercise may be common [73]. The principal diagnostic tool is the immunostaining of muscle that reveals a significantly reduced signal with antibodies recognizing the glycosylated epitopes of α-dystroglycan. There is a correlation between the reduced α-dystroglycan staining and clinical course in individuals with mutations in POMT1, POMT2, and POMGnT1, but this is not always the case in FKN and FKRP gene mutations [74].

Recently, Hara et al. [75**] have reported a missense mutation in the dystroglycan gene in an LGMD patient with cognitive impairment. This substitution interferes with LARGE-dependent maturation of phosphorylated O-mannosyl glycans on α-dystroglycan affecting its binding to laminin.

LGMD2J (titin gene)
A homozygous mutation in the C terminus of titin (FINmaj 11 bp deletion/insertion) causes LGMD2J [76]. Titin is the giant sarcomeric protein that forms a continuous filament system in the myofibrils of striated muscle, with single molecules spanning from the sarcomeric Z-disc to the M-band [77]. Other ‘titinopathic’ clinical pictures are tibial muscular dystrophy (TMD, Udd myopathy) or more severe cardiac and muscular phenotypes.

CAPN3 binds M-band titin at is7 within the region affected by the LGMD2J mutations and shows a secondary deficiency in LGMD2J muscle [28]. Interactions with titin may protect CAPN3 from autolytic activation and removal of the CAPN3 protease reverses the titin myopathy [78].

Identification of the French nonsense mutation (Q33396X) located in Mex6 seems to cause a milder phenotype than the typical FINmaj mutation [77]. Due to the huge gene size, there is limited availability of genetic tests for titin defects, based on mutation-specific genotyping.

LGMD2L
Recessive mutations in the putative calcium-activated chloride channel Anoctamin 5 (ANO5) cause proximal LGMD2L and distal MMD3 muscular dystrophies [79*,80,81]. ANO5 represents a relatively common cause of adult onset muscular dystrophy in England, for the regional prevalence of the c.191dupA mutation. Lower limb involvement is atrophic and often asymmetric, with
high serum CK, the weakness is generally slowly progressive. Sequencing of all exons is necessary.

Upcoming molecular diagnoses

It is generally accepted that, after the results from a biopsy and protein testing, a specific genetic test is performed to confirm and complete the LGMD diagnosis [2]. There is, however, the prospect that next-generation sequencing (NGS)-based targeted exome sequencing [82] will reverse this order, making affordable a universal DNA test that screens for all the neuromuscular disease genes. In this case many nonpenetrant mutations will be discovered and the interpretation of the results will be crucial. Universal tests are already in use for quantitative muscle biopsy. Whole genome homozygosity mapping has been proposed for the mapping of consanguineous cases of LGMD2 [84,85]. Sequence analysis of mRNA is required for the diagnosis of more than 10% of mutations, as deep intronic or elusive exonic variations may disrupt the correct splicing; this requires a muscle biopsy, even if mRNA may be used from blood (only LGMD2A,B) or perioral muscle fibers (skin biopsy) [86].

Upcoming therapies

Treatment of LGMD remains palliative and supportive. Physiotherapy to prevent joint deformities and promote walking is recommended. A passive stretching physical therapy programme should be instituted early, soon after diagnosis. The use of knee–ankle–foot orthoses at bedtime is recommended to prevent contractures.

The benefit of steroids has been reported in some types of LGMD, including LGMD2D [87], LGMD2I [88], and LGMD2L [89]. A double-blind, placebo-controlled study of deflazacort in LGMD2B/Miyoshi myopathy is in progress (http://clinicaltrials.gov).

An alpha-sarcoglycan gene expression in two of three LGMD2D subjects was obtained for 6 months by adeno-associated virus-mediated (AAV) gene transfer to the extensor digitorum brevis muscle [90,91]. Although a systemic AAV gene therapy is effective in terms of dystrophic muscles and restores the dystrophic muscles and restore dystrophin expression in SCID/BIAJ mice [98].

Conclusion

Advances in the knowledge of LGMDs have been made and 24 different LGMDs have been so far recognized. Next generation sequencing technologies promise a revolution in diagnostics and characterization of additional LGMD genes. Novel systemic therapies that have been effective in the different animal models will be translated into clinical trials.

Acknowledgement

The authors thank Mr Jon Cole for his assistance in drafting this article.

Conflicts of interest

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 513).


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