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(54)【発明の名称】精神分裂病の診断のための新規ペプチド

(57)【要約】
非精神分裂病者から得られた体液試料への結合より実質的に高いレベルで、精神分裂病患者から得られた体液試料に結合する短いペプチドが提供される。ペプチドは、１０アミノ酸以下の長さであり、その末端の１つで少なくとも１つの陽性荷電アミノ酸を含む少なくとも５つのアミノ酸の連続的配列を含む。従って、精神分裂病者で高レベルで見いだされる自己抗体の推定結合部位である、提供されるペプチドは、精神分裂病の診断に有用である。

【特許請求の範囲】
【請求項１】
非精神分裂病者から得られた体液試料への結合より実質的に高いレベルで、精神分裂病患者から得られた体液試料に結合するペプチドであって、該ペプチドは１０アミノ酸（ａ．ａ．）以下の長さであり、以下の配列：
ｉ．　ＬＶＶＧＬＣＫ（配列番号１）
ii．　ＫＬＶＶＧＬＣ（配列番号２）
iii．　ＬＶＶＧＬＭＫ（配列番号３）
iv．　ＫＬＶＶＧＬＭ（配列番号４）
のいずれか１つに含まれる少なくとも５つのアミノ酸の連続的配列を含み；
該連続的配列は、該配列の１つの末端に少なくとも１つの陽性荷電ａ．ａ．を含み；そして該ペプチドは、該連続的配列の陽性荷電ａ．ａ．である少なくとも１つの陽性荷電ａ．ａ．または少なくとも１つの追加の陽性荷電ａ．ａ．を、その末端に含むペプチド；
または、１０ａ．ａ．以下の長さである該ペプチドの類似体で、ここで該連続的配列の２つ以下のａ．ａ．は保存的に置換され、ペプチドの結合特性を基本的に保持している類似体。
【請求項２】
非精神分裂病者から得られた体液試料への結合より実質的に高いレベルで、精神分裂病患者から得られた体液試料に結合するペプチドであって：
ｉ．　ＬＶＶＧＬＣＫ（配列番号１）
ii．　ＫＬＶＶＧＬＣ（配列番号２）
iii．　ＬＶＶＧＬＭＫ（配列番号３）
iv．　ＫＬＶＶＧＬＭ（配列番号４）
よりなる群から選択されるペプチド、
または、２つ以下のａ．ａ．が保存的に置換されている類似体であって、ペプチドの結合特性を基本的に維持している類似体。
【請求項３】
非精神分裂病者から得られた体液試料への結合より実質的に強く、精神分裂病患者から得られた体液試料に結合する、アミノ酸配列ＬＶＶＧＬＣＫ（配列番号１）を有するペプチド。
【請求項４】
非精神分裂病者から得られた試料への結合より実質的に強く、精神分裂病患者から得られた体液試料に結合するペプチドであって、アミノ酸ＬＶＶＧＬＣＫ（配列番号１）を有するペプチドへに特異的に結合することができる抗体に結合するペプチド。
【請求項５】
アミノ酸配列ＫＬＶＶＧＬＣ（配列番号２）を有する請求項４のペプチド。
【請求項６】
アミノ酸配列ＬＶＶＧＬＭＫ（配列番号３）を有する請求項４のペプチド。
【請求項７】
アミノ酸配列ＫＬＶＶＧＬＭ（配列番号４）を有する請求項４のペプチド。
【請求項８】
個人の精神分裂病を診断するための測定法であって：
（ａ）該個人から、血液試料、そのＰＡＡ含有画分、または血小板から流れ出たＰＡＡを含有する画分である、体液試料を得る工程；
（ｂ）該試料に、１０以下のアミノ酸（ａ．ａ．）の長さであって、以下の配列：
ｉ．　ＬＶＶＧＬＣＫ（配列番号１）
ii．　ＫＬＶＶＧＬＣ（配列番号２）
iii．　ＬＶＶＧＬＭＫ（配列番号３）
iv．　ＫＬＶＶＧＬＭ（配列番号４）
のいずれか１つに含まれる少なくとも５つのアミノ酸の連続的配列を含むペプチド（該連続的配列は、該配列の１つの末端に少なくとも１つの陽性荷電ａ．ａ．を含み；そして該ペ

プチドは、該連続的配列の陽性荷電ａ.ａ.である少なくとも１つの陽性荷電ａ.ａ.または少なくとも１つの追加の陽性荷電ａ.ａ.を、その末端に含む）；
または、１０ａ.ａ.以下の長さであり、ここで該連続的配列の２つ以下のａ.ａ.は保存的に置換され、ペプチドの結合特性を基本的に保持している、該ペプチドの類似体を、接触させる工程；
（ｃ）試料へのペプチドの結合レベルを測定する工程（ここで、非精神分裂病者から得られた試料へのペプチドの結合レベルより実質的に高い結合レベルは、試験された個人が、精神分裂病を有する可能性が高いことを示す）
を含んでなる上記方法。
【請求項９】
工程（ｂ）のペプチドは：
ｉ.　ＬＶＶＧＬＣＫ（配列番号１）
ii.　ＫＬＶＶＧＬＣ（配列番号２）
iii.　ＬＶＶＧＬＭＫ（配列番号３）
iv.　ＫＬＶＶＧＬＭ（配列番号４）
よりなる群、または２つ以下のａ.ａ.は保存的に置換され、ペプチドの結合特性を基本的に保持している、該ペプチドの類似体、
から選択される請求項８の方法。
【請求項１０】
工程（ｂ）のペプチドはアミノ酸配列ＬＶＶＧＬＣＫ（配列番号１）を有する請求項８の方法。
【請求項１１】
工程（ｂ）のペプチドは、アミノ酸配列ＬＶＶＧＬＣＫ（配列番号１）を有するペプチドに結合する抗体に結合するようなもの、または２つ以下のａ.ａ.が保存的に置換されているその類似体である請求項８の方法。
【請求項１２】
精神分裂病の診断で有用なキットであって、その上に固定化された請求項１の１つ以上のペプチドを含む支持体、抗ヒト免疫グロブリン（ｈＩｇ）抗体またはその断片、検出測定法（こうして該ペプチドは、試験試料中に存在する抗体に結合する）を有する試薬、ならびにその使用説明書を含む上記キット。
【請求項１３】
抗ｈＩｇ抗体は検出可能なマーカーと複合体を形成している請求項１２のキット。
【請求項１４】
キットは、抗ｈＩｇ抗体の代わりに、試験試料中に存在する１次抗体に対する１つ以上の非結合ペプチドを含み、該ペプチドは検出可能なマーカーと複合体を形成している請求項１２のキット。
【請求項１５】
個人の精神分裂病を診断するための診断用組成物の製造のための、請求項１の任意のペプチドの使用。
【請求項１６】
少なくとも１つの他の診断測定法により測定される、個人の精神分裂病の高い確率を確認するために使用される請求項８の測定法。
【発明の詳細な説明】
【技術分野】
【０００１】
本発明は、精神分裂病の診断のための測定法に関し、この測定法で使用するための新規ペプチドを提供する。
【背景技術】
【０００２】
以下は、本発明の背景をよりよく理解するための刊行物のリストである。上記先行技術を

本明細書で認めることは、これらの技術が、請求の範囲に記載の本発明の特許性に関連することを示すものではない。

【０００３】

Carpenter, W.T. と Buchanan, R.W.: Review, New Engl. J. Med.,330:681-690, 1994.
Deckmann, M., Shinitzky, M., Leykin, I., Cheng, D., Guy, J., Avnon, M., Salganik, I., Amiri, Z., Schlossberg, A., Leibu, E. および Rafael, C.:The Italian J. Psychiatr. Behav. Sci.,6:29-34, 1996.
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Shinitzky, M.とDeckmann, M.、「精神分裂病への罹り易さの診断」、米国特許第6,008,001号、1999.
Shinitzky, M.とDeckmann, M.、「皮膚反応に基づく精神分裂病の測定法」、WO99/30163、1999.
Shinitzky, M.とDeckmann, M.、「新しいペプチドに基づく精神分裂病の診断のための測定法」、WO99/51725、1999.

【０００４】

精神分裂病は、種々の聴覚性幻覚、パラノイア、妄想、緊張病、奇怪な行動、および感情的引きこもりのような種々の精神症状を包含する一連の疾患である。精神分裂病は、全人口の約１％をおそい、社会に対するその経済的ならびに社会的負担は莫大である。この疾患の発症は若年であり、従って患者は、一生の医学的かつ精神医学的監督が必要である。従って精神分裂病は、先進国の最も費用のかかる疾患の１つとされている（Carpenterら、1994）。

精神分裂病に関連した一般的なパラメータは同定されておらず、従って国際的に認められたこの疾患の診断は、今日も精神医学的評価にのみ基づく。精神分裂病に関連する公知の危険因子は、遺伝的素因、冬季の出産、および妊娠または出産時の合併症である。以後の自己免疫反応に関連するウイルスおよび／または細菌感染症が、精神分裂病の発症増加の原因因子であると提唱されている（DeLisiら、1987）。

精神分裂病は、自己免疫過程が関与することが証明されており、最近、精神分裂病患者で血小板に対する自己抗体と細胞障害性Ｔ細胞が証明された（Shinitzky, 1991, Deckmann, 1996, Shinitzky, 1999, 米国特許第６，００８，００１号）。精神分裂病患者の細胞障害性Ｔ細胞反応は皮膚反応で評価され、ここでは、ほとんどの精神分裂病患者は、自己血小板に対して陽性に反応し、非精神分裂病の試験したほんのわずかの人のみが、この試験で陽性に反応した（Shinitzky, 1999、ＷＯ９９／３０１６３）。

さらに、血小板に対する自己抗体レベルの上昇が精神分裂病患者で観察されたが、躁うつ病、うつ病、人格障害、および分裂情動性障害患者では観察されなかった（Shinitzky, 1999、およびDeckmann, 1996）。

【０００５】

本発明者らの以前の研究において、精神分裂病患者の体液中に高レベルで存在する自己抗体に結合するいくつかのタンパク質が同定された（Shinitzky, 1999、ＷＯ９９／５１７２５号）。これらのタンパク質は、精神分裂病患者の自己抗体由来の精製した血小板（ＰＡＡ）に反応したが、精神分裂病者および非精神分裂病者の血漿または血液試料を区別できなかった。これらのタンパク質の１つ（酵素エノラーゼ）を酵素処理すると、非精神分裂病者の血漿試料より精神分裂病患者の血漿試料に実質的に強く結合する断片が得られた。この断片に基づいて、いくつかの追加のペプチドを合成し、精神分裂病者のＰＡＡに対して強い結合性を有するものを単離した。これらのペプチドの抗原性ペプチドの構造は、コンピュータープログラムを使用して、疎水性コアと、約２つの陽性荷電を有する延長部分とを含む環状構造である３次元エピトープであると予測された。免疫学的研究は、この

ペプチドの酸化環状型のみが抗血漿自己抗体と反応性であることを示した。これらの合成したペプチドは、少なくとも１７個のアミノ酸（ａ.ａ.）を含有した。

【発明の開示】

【発明が解決しようとする課題】

【０００６】

本発明において、先行技術（ＷＯ９９／５１７２５）に記載のペプチド配列は、精神分裂病患者の体液中に高レベルで存在する自己抗体により強く結合することができ、非精神分裂病者の体液中のものにはより弱く結合するかまたは全く結合せず、これらのペプチドは、そのような自己抗体の天然の結合部位ではあり得ないことがわかった。これは、この配列が、これらの起源であるタンパク質（酵素エノラーゼ）の表面に露出していないという事実による。４０２位の単一のアミノ酸アルギニンを除くと、残りのアミノ酸はタンパク質内に埋まっている（図１を参照）。さらに公知のように、抗体結合部位は通常約５～８個のａ.ａ.を含み、これらの各ペプチドは、必ずしも結合部位ではない１７個のａ.ａ.を含んだ。

【０００７】

従って本発明において、精神分裂病患者中で高レベルに存在する自己抗体の天然の結合部位であるペプチドが、そのような自己抗体に対してより高感度で特異的に結合し、従って精神分裂病の診断において有用であるという理解に基づいて、３次元モデリングを使用して、エノラーゼの表面上の同等の部位の同定を試みた。エノラーゼの表面を探索するための３次元構造を使用すると、Ｌ４１２、Ｌ１８３、Ｌ４０９、Ｌ４０６、Ｐ４００およびＡ４０１と呼ぶ中性アミノ酸のクラスターを囲むＲ４１４、Ｒ１８４、Ｋ１９４およびＲ４０２と呼ぶ４つの陽性荷電アミノ酸からなる推定エピトープ（図２を参照）が同定された。そのような推定エピトープを有するペプチドは、本発明に従って提供される。

【課題を解決するための手段】

【０００８】

すなわち、第１の態様において本発明は、非精神分裂病者から得られた体液試料への結合より実質的に高いレベルで、精神分裂病患者から得られた体液試料に結合するペプチドであって、該ペプチドは１０アミノ酸（ａ.ａ.）以下の長さであり、以下の配列：

ｉ.　ＬＶＶＧＬＣＫ（配列番号１）

ii.　ＫＬＶＶＧＬＣ（配列番号２）

iii.　ＬＶＶＧＬＭＫ（配列番号３）

iv.　ＫＬＶＶＧＬＭ（配列番号４）

のいずれか１つに含まれる少なくとも５つのアミノ酸の連続的配列を含み；

該連続的配列は、該配列の末端に少なくとも１つの陽性荷電ａ.ａ.を含み；そして該ペプチドは、該連続的配列の陽性荷電ａ.ａ.である少なくとも１つの陽性荷電ａ.ａ.または少なくとも１つの追加の陽性荷電ａ.ａ.を、その末端に含むペプチド；

または、１０ａ.ａ.以下の長さである該ペプチドの類似体で、ここで該連続的配列の２つ以下のａ.ａ.は保存的に置換され、ペプチドの結合特性を基本的に保持している類似体

を提供する。

【０００９】

本発明の「実質的に高レベルの結合」は、当該分野で公知の任意の結合測定法、例えば後述されるものを使用して測定され、適当な統計的検定、例えばスチューデントｔ検定により調べると、精神分裂病患者から得られた試料へのペプチドの結合の測定されたレベルは、非精神分裂病患者から得られた試料への同じペプチドの結合の測定されたレベルより有意に高い。

用語「連続的配列」は、その末端に陽性荷電ａ.ａ.を含む、配列番号１～４の任意の配列の５～７個のａ.ａ.の中断されない配列に関する。陽性荷電ａ.ａ.は好ましくはリジン（配列中ではＫとして示す）であるが、アルギニン（Ｒ）またはヒスチジン（Ｈ）でもよい。

【００１０】

連続的配列は、より長い最大１０ａ.ａ.のペプチドの一部でもよく、連続的配列はペプチド中のどこにあってもよい。ペプチドが７を超えるａ.ａ.を含み、連続的配列がペプチドの１つの末端にある場合、大きいペプチドがその１つの末端に陽性荷電ａ.ａ.を含むように、陽性荷電ａ.ａ.は配列（これは、より長いペプチドの追加のａ.ａ.に結合していない）の開いた末端にある。連続的配列がペプチドの真ん中にある場合、ペプチドは、連続的配列の陽性荷電ａ.ａ.以外に、その１つの末端に少なくとも１つの追加の陽性荷電ａ.ａ.を含む。

上記ペプチドの類似体もまた、本発明の範囲内にある。そのような類似体は、上記連続的配列の１つと同じ配列を有する少なくとも５ａ.ａ.を含む１０以下のａ.ａ.を含むが、１つまたは２つのａ.ａ.は保存的に置換（この用語は後に定義される）される。類似体はまた、その末端に少なくとも１つの陽性荷電ａ.ａ.を含み、ペプチドの活性（この用語は後に定義される）を基本的に維持する。

【００１１】

用語「結合特性を基本的に維持する」は、同じ結合測定法で測定する時、試験試料への結合レベルが、同じ試験試料へのペプチドの結合レベルの少なくとも５０％、好ましくは７０％、最も好ましくは９０％または１００％を超えるペプチドを意味する。

【００１２】

好適な実施態様において本発明は、非精神分裂病者から得られた体液試料への結合より実質的に高いレベルで、精神分裂病患者から得られた体液試料に結合するペプチドであって：

ｉ.　ＬＶＶＧＬＣＫ（配列番号１）
ii.　ＫＬＶＶＧＬＣ（配列番号２）
iii.　ＬＶＶＧＬＭＫ（配列番号３）
iv.　ＫＬＶＶＧＬＭ（配列番号４）

よりなる群から選択されるペプチド、

または、２つ以下のａ.ａ.が保存的に置換されている類似体であって、ペプチドの結合特性を基本的に維持している類似体を提供する。

最も好適な実施態様において、非精神分裂病者から得られた体液試料への結合より実質的に強く、精神分裂病患者から得られた体液試料に結合する、アミノ酸配列ＬＶＶＧＬＣＫ（配列番号１）を有するペプチドが提供される。

【００１３】

本発明のさらなる態様において、非精神分裂病者から得られた試料への結合より実質的に強く、精神分裂病患者から得られた体液試料に結合するペプチドであって、アミノ酸ＬＶＶＧＬＣＫを有するペプチドに特異的に結合することができる抗体に結合するペプチドが提供される。そのようなペプチドのいくつかの非限定例は：

ｉ.　ＫＬＶＶＧＬＣ（配列番号２）
ii.　ＬＶＶＧＬＭＫ（配列番号３）
iii.　ＫＬＶＶＧＬＭ（配列番号４）

または、２つ以下のａ.ａ.が保存的に置換されている類似体であって、ペプチドの結合特性を基本的に維持している類似体である。

特定のアミノ酸（ａ.ａ.）を示すために上記および本説明を通して使用される文字は、ＩＵＰＡＣ－ＩＵＢ生化学命名委員会（Biochemical Nomenclature Commission）により推奨される一文字ａ.ａ.記号に一致する。

【００１４】

本発明のペプチドは、精神分裂病患者の体液試料中に高レベルで存在する自己抗体の推定の天然の結合部位であり、その高純度と高活性のために、これらのペプチドは精神分裂病の診断に非常に有用である。すなわち、さらなる態様において本発明は、個人の精神分裂病を診断するための測定法であって：

（ａ）該個人から、血液試料、その血小板関連抗体（ＰＡＡ）含有画分、または血小板から流れ出たＰＡＡを含有する画分である、体液試料を得る工程；

（ｂ）該試料に、１０以下のアミノ酸（ａ.ａ.）の長さであって、以下の配列：
ｉ.　ＬＶＶＧＬＣＫ（配列番号１）
ii.　ＫＬＶＶＧＬＣ（配列番号２）
iii.　ＬＶＶＧＬＭＫ（配列番号３）
iv.　ＫＬＶＶＧＬＭ（配列番号４）
のいずれか１つに含まれる少なくとも５つのアミノ酸の連続的配列を含むペプチド（該連続的配列は、該配列の１つの末端に少なくとも１つの陽性荷電ａ.ａ.を含み；そして該ペプチドは、該連続的配列の陽性荷電ａ.ａ.である少なくとも１つの陽性荷電ａ.ａ.または少なくとも１つの追加の陽性荷電ａ.ａ.を、その末端に含む）；
または、１０ａ.ａ.以下の長さであり、ここで該連続的配列の２つ以下のａ.ａ.は保存的に置換され、ペプチドの結合特性を基本的に保持している、該ペプチドの類似体を、接触させる工程；
（ｃ）試料へのペプチドの結合レベルを測定する工程（ここで、非精神分裂病者から得られた試料へのペプチドの結合レベルより実質的に高い結合レベルは、試験された個人が、精神分裂病を有する可能性が高いことを示す）
を含んでなる上記方法を提供する。

【００１５】

好適な実施態様において、工程（ｂ）のペプチドは：
ｉ.　ＬＶＶＧＬＣＫ（配列番号１）
ii.　ＫＬＶＶＧＬＣ（配列番号２）
iii.　ＬＶＶＧＬＭＫ（配列番号３）
iv.　ＫＬＶＶＧＬＭ（配列番号４）
よりなる群、または２つ以下のａ.ａ.は保存的に置換され、ペプチドの結合特性を基本的に保持している、該ペプチドの類似体から選択される。

【００１６】

好適な実施態様において工程（ｂ）のペプチドは、アミノ酸配列ＬＶＶＧＬＣＫを有する。
さらなる態様において工程（ｂ）のペプチドは、アミノ酸配列ＬＶＶＧＬＣＫを有するペプチドに結合する抗体に結合するようなもの、またはその類似体である。
個人の精神分裂病の診断のための診断用組成物の調製のための、上記および後述の本発明のペプチドおよびその類似体の使用もまた、本発明の範囲内である。

【００１７】

さらなる態様において本発明は、上記測定法で有用なキットであって、その上に固定化された本発明の１つ以上のペプチドを含む支持体、抗ヒト免疫グロブリン（ｈＩｇ）抗体またはその断片、試験試料中に存在する抗体に結合する検出可能なマーカーに結合した１つ以上の非結合ペプチド、検出測定法を実施するのに必要な試薬（ここで該ペプチドは、試験試料中に存在する抗体に結合する）、ならびにその使用説明書を含むキットを提供する。
試験試料への本発明のペプチドの結合の検出が、抗ｈＩｇ抗体による場合、抗ｈＩｇ抗体は検出可能なマーカーに結合してもよく、あるいは、キットは、１次抗体に対する第２の型の抗体を含んでもよく、第２抗体は検出可能なマーカーに結合している。

【００１８】

ある実施態様において試験試料への本発明のペプチドの結合は、検出可能なマーカーと複合体を形成した第２の非結合ペプチドを使用して検出され、この第２のペプチドは、試験試料中に存在する抗体に結合することができる。この実施態様において検出は、２重抗原サンドイッチテキストに行われ、これは、１工程測定法または２工程測定法として行われる。検出が２重抗原サンドイッチテキスト測定法として行われる場合、本発明のキットは、抗ヒト免疫グロブリン抗体の代わりに検出可能なマーカーに結合したそのようなペプチドを含むであろう。
本発明の測定法は、個人の精神分裂病の高確率を検出するための単一の検査として使用す

ることができる。しかし本発明のさらなる態様において、ペプチドと測定法は、確認診断手段として使用してもよい。すなわち例えば、今日まで使用されている方法（上記のような、主に精神医学的評価）により個人で精神分裂病の高い可能性が測定されると、これは、本発明の測定法を使用して再確認（または、再評価）されるであろう。

【００１９】

（発明の詳細な説明）

本発明は、短い高度に精製された高活性のペプチドを提供し、これは、精神分裂病患者中に高レベルで存在する自己抗体が結合するペプチドの推定の天然エピトープを含む。これらのペプチドの短い長さ（７～１０アミノ酸）とその構造（自己抗体が結合することを可能にするように、エノラーゼ酵素の表面に露出することができる）は、これらのペプチドを、精神分裂病の診断に最も有用なものとする。すなわち、先行技術（ＷＯ９９／５１７２５）のペプチドは、非精神分裂病者と比較して精神分裂病患者中に高レベルで存在する自己抗体に結合することができるが、上記特徴を有する本発明のペプチドは、精神分裂病を高い確率で有する個人をより効果的に検出することを可能にする。

【００２０】

種々の抗体への本発明のペプチドの結合活性は、それ自体公知の任意の方法（例えば、ＥＬＩＳＡまたはウェスタンブロッティング）により測定される。例えば、試験ペプチドは、ポリアクリルアミドゲル電気泳動に付し、ＰＶＤＳ膜にブロッティングし、次に試験試料の体液試料と反応させ、非精神分裂病者から得られる試料との反応性について比較することにより、抗体への結合活性を分析することができる。

本発明のペプチドのＰＡＡへの結合の程度は、当該分野で公知の任意の検出系（例えば、検出可能なマーカーに結合した、ヒト免疫グロブリンまたはその断片に対する抗体）を使用して測定することができる。マーカーは、放射性基、蛍光性基、検出可能な生成物を生じる反応を触媒することができる酵素、アビジンにより検出することができるビオチン基などでもよい。

好適な実施態様において本発明のペプチドの試験試料への結合の程度は、酵素免疫測定法（後述のように、ペプチドはビオチンで標識され、ストレプトアビジン被覆チューブに結合される）を使用して測定される。

【００２１】

本発明において、ペプチド中の陽性荷電の位置は、ペプチドの最初でもまたは最後でもよいことがわかっている。これは、ペプチドが開環型で存在することを示す。さらに、システインをメチオニンで置換してもペプチドの活性が保持されたため、ペプチドの配列においてシステインはあまり重要ではないことを示す。すなわち上記したように、本発明のペプチド内に含有されるａ.ａ.の連続的配列は、その末端に陽性荷電ａ.ａ.を含む。連続的配列が全ペプチドの末端の１つにあるなら、この陽性荷電ａ.ａ.は、全ペプチドの１つの末端にある。あるいは連続的配列が全ペプチドの末端にないなら、全ペプチドは、その末端の１つに追加の陽性荷電を含有するであろう。

【００２２】

抗体結合部位は通常、少なくとも５つのアミノ酸を含むことは公知である。本発明のペプチドとその類似体は、少なくとも５つのアミノ酸を含む。この５つのアミノ酸は、本発明の連続的配列の１つで連続的に現れるものである。

ペプチドの結合特性を維持するために、本発明の類似体は、保存的に置換である２以下のａ.ａ.置換を有する。これらは、あるクラスのアミノ酸が同じクラスのアミノ酸により置換される置換であり、クラスは、一般的な物理化学的アミノ酸鎖の性質（例えば、電荷、サイズまたは疎水性）により規定される。同じクラスのアミノ酸は、自然界で見つかる相同的なタンパク質中の高い置換頻度（例えば、標準物質Dayhoff頻度交換マトリックスにより測定すると）を特徴とする。

【００２３】

すなわち、例えば本発明のペプチドのアミノ酸配列の最初の位置に存在するロイシンは、同じファミリーのアミノ酸に属するアミノ酸グリシンまたはバリンにより保存的に置換し

ても、ペプチドの結合活性を変化させることはない。本発明のペプチドの末端の陽性荷電ａ.ａ.は、別の陽性荷電ａ.ａ.により保存的に置換される。例えば、「細胞の分子生物学（Molecular Biology of the Cell）」Alberts B.ら編、ガーランドパブリッシング（Garland Publishing, In.c）、ニューヨークとロンドン、第２版、１９８９年、５４～５５頁に記載のように、ファミリーへのアミノ酸の公知の分類に従って、ペプチドのアミノ酸がどのアミノ酸で置換できるかを決定することは、当業者には困難ではないであろう。

【００２４】

本発明のペプチドはまた、化学修飾してもよい。そのような化学修飾ペプチドにおいて、少なくとも１つのアミノ酸残基は、翻訳後修飾のような自然のプロセスまたは当該分野で公知の化学修飾法により修飾される。化学修飾の例は、アセチル化とグリコシル化、グリコサミン－グリシン化、ＡＤＰ－リボース化、脂質または脂質誘導体の共有結合、メチル化、ミリスチル化、ＰＥＧ化（pegylation）、リン酸化などがある。化学修飾は、ペプチドのアミノ末端またはカルボキシ末端でもよい。

本発明のペプチドはさらに、例えば、ペプチドのアミノ側またはカルボキシ側に共有結合した巨大分子担体基のような非ペプチド成分を有してもよい。そのような担体は例えば、ポリエチレングリコール、炭水化物または脂質脂肪酸結合体がある。

上記のすべては、本発明のペプチドの安定性、バイオベイラビリティ、または活性を上昇させる、本発明のペプチドの変化を説明する。

【００２５】

本発明のペプチドは、より長いタンパク質の酵素的消化（例えば、クロストラパインを使用して）または化学的（ＣＮＢｒ）消化により得られる。そのような場合、生じるペプチドは、当該分野で公知の方法（例えばＲＰ－ＨＰＬＣ）により分離され、分離されたペプチドは次に、配列決定（Eurosequence b.v.（Nijenborgh 4:9749 Gronigern; オランダ））用に使用され、上記の抗体への結合活性について分析される。

本発明のペプチドの短い長さ（５～１０ａ.ａ.）は、これらを当該分野で公知の方法（例えばEurosequence b.v.（詳細は後述の例を参照）による１０μmolスケールでアビメド（Abimed）５２２上）による合成のための非常に良好な候補にしている。新たに合成されたペプチドの結合活性は、上記の任意の測定法を使用して測定されるであろう。

【００２６】

本発明のペプチドが短く、精製されかつ活性ペプチドであるという事実に基づく本発明の他の利点は、試験すべき個人の「体液」試料は、入手し調製することが比較的容易な血漿または血清試料でもよいことである。しかし、血小板多血漿を得てそこからＰＡＡを単離するような、当該分野で公知の任意の方法により試料から得られるＰＡＡ含有画分である、試験される個人から得られる血液試料について、本発明の測定法を行うことが有利な場合がある。本発明において試料はまた、試験される個人から得られる任意の他の体液試料でもよく、全血試料、またはＰＡＡを含有する任意の他の体液試料（例えば、唾液、髄液など）でもよい。化学分析ならびに本発明の免疫活性測定法は、アミノ酸配列ＬＶＶＧＬＣＫを有するペプチドが最も高い合成純度を有し、自然にダイマーを形成し、こうしてストレプトアビジンの結合部位あたりのエピトープを２倍にし、ペプチドへのＰＡＡの結合確率を上昇させることを証明した。従って本発明において、上記配列を有するペプチドまたはこの配列を含むペプチドは、本発明の精神分裂病の診断における使用に好適である。

【００２７】

実施例

本発明を、図面を参照して以下の非限定例に従って説明する。

材料と方法

１．患者と対照人　－　３９人の精神分裂病患者がこの試験に参加した。以下のパラメータを記録した：性、年齢、精神分裂病の持続期間、入院回数、教育年数、および精神医学的状態（ＰＡＮＳＳ）。地方の血液銀行から５０個の血漿試料を得た。

２．血漿　－　患者と対照被験体から、ヘパリンを凝固剤として使用して静脈血を採取した。遠心分離（４０００ｇ、１５分、４℃）後に血漿を得た。

３．ペプチド合成とビオチン標識 － Eurosequence b.v.（Nijenborgh 4:9749 Groningern; オランダ）により１０μmolスケールでアビメド（Abimed）５２２上で、ペプチドを合成し、６つの炭素スペーサーを含有するビオチンでアミノ末端で標識した。ペプチド純度はＲＰ－ＨＰＬＣによりルーチン法により測定し、必要と判断した時は、レーザー脱着質量スペクトル法（laser desorption mass spectroscopy）により測定した。ペプチドを、１ｍｌの水／ＤＭＦ／ＤＭＳＯ（１：１：１、ｖ／ｖ／ｖ）にルーチン法で溶解した。
４．ラインブロット － ペプチドをＰＶＤＦまたはニトロセルロース膜上にラインブロットした。膜のストリップを、０．５ｍｌの緩衝液（２００ｍＭトリス；０．３％カゼイン；２００ｍＭ ＫＣｌ；１０，６ｍＭ フェノール；２．１ｍＭ $CaCl_2$；０．０１％トリトンＸ－１００；ｐＨ８．５）中０．５ｍｌの血漿試料を用いて、室温でバイオラッド（BioRad）インキュベーションアレイ中で静かに振盪して一晩インキュベートした。ＰＢＳで３回洗浄後、ストリップを西洋ワサビペルオキシダーゼ結合抗ヒトＦｃ（ヤギ）（シグマ（Sigma）；希釈１：１００）で、静かに振盪しながら室温で２時間プローブ結合させた。Ｆａｓ－ＤＡＢ（登録商標）または４－クロロ－ナルトール（シグマ（Sigma））を、結合抗体を検出するための発色試薬として使用した。
５．酵素免疫定量法 － ストレプトアビジン被覆チューブ（ベーリンガーマンハイム（Boehringer Mannheim）；８０ｎＭ ストレプトアビジン／チューブ）を、１ｍｌのＰＢＳ中１０倍過剰のビオチン標識ペプチドで４℃で３日間被覆し、２ｍｌの１Ｍ ＮａＣｌで２回洗浄し、２ｍｌの水で１回洗浄し、乾燥し、使用するまで－１８℃で真空下で保存した。検査のために、０．０５ｍｌの試料と１ｍｌのインキュベーション緩衝液（６０ｍＭクエン酸；９０ｍＭ Na_2HPO_4；１６８ｍＭ ＮａＯＨ；２００ｍＭ ＮａＣｌ；ｐＨ７．７）を、ペプチド標識チューブに加え、３７℃で１時間振盪無しでインキュベートした。蒸発を防ぐためにチューブにカバーをした。次にチューブを、２ｍｌの１Ｍ ＮａＣｌ溶液で１５分静かに振盪しながら３回洗浄した。１ｍｌの西洋ワサビペルオキシダーゼ結合抗ヒトＦｃ（ヤギ）（シグマ（Sigma））を、ＰＯＤ緩衝液（２００ｍＭトリス；０．３％カゼイン；２００ｍＭ ＫＣｌ；１０，６ｍＭ フェノール；２．１ｍＭ $CaCl_2$；０．０１％トリトンＸ－１００；ｐＨ８．０）で１：２０００希釈して加え、振盪無しで３７℃で１５分インキュベートした。２ｍｌの１Ｍ ＮａＣｌ溶液で４回洗浄後、１ｍｌのテトラメチルベンジジン（ＴＭＢ）液体基質系（登録商標）（シグマ（Sigma））を加えて発色反応を開始した。１ｍｌの０．５Ｍ H_2SO_4を加えて反応を停止させた。４５０ｎｍで分光学的に吸光度を読んだ。

【実施例１】

【００２８】

ａ.ａ.配列：

（ａ） ＬＶＶＧＬＣＫ
（ｂ） ＫＬＶＶＧＬＣ
（ｃ） ＬＶＶＧＬＭＫ
（ｄ） ＫＬＶＶＧＬＭ

の１つを有する本発明の４つのペプチドをビオチン標識し、上記したようにストレプトアビジン被覆チューブ上に被覆した。血漿試料のプールを５人の精神分裂病患者から調製し、血漿の追加のプールを５人の非精神分裂病者から調製した。

【００２９】

結果：

本発明の４つのすべてのペプチドは、非精神分裂病者から得られた対照血漿試料への結合より強く、精神分裂病患者からの血漿プールに結合した（上記酵素免疫定量法で測定し、これは、対照血漿試料のペプチド試料は０．５ＯＤ、そして精神分裂病由来血漿プールのペプチド試料は１．６ＯＤ）。

【実施例２】

【００３０】

ペプチドＬＶＶＧＬＣＫを使用した精神分裂病の診断

本発明の４つのペプチドのうち、最も高い合成純度は、ペプチドＬＶＶＧＬＣＫで見いだされた。さらにこのペプチドは、自然にダイマーを形成し、こうしてストレプトアビジンの結合部位あたりのエピトープを倍にした。
従ってビオチン－ＬＶＶＧＬＣＫを用いて以下のように実験を行った：ペプチドをビオチン標識し、上記したようにストレプトアビジン被覆チューブ上に被覆した。３９人の精神分裂病患者と５０人の対照の非精神分裂病者の血漿試料を、被覆ペプチドで試験し、上記したように結合レベルを酵素免疫定量法により測定した。
図３に示すように、ビオチン化ペプチドＬＶＶＧＬＣＫは、精神分裂病患者から得られた血漿試料（黒四角）に平均と標準偏差１．４７±０．６５で強く結合し、これに対して非精神分裂病者から得られた血漿試料（白四角）に平均と標準偏差０．４６±０．２１で結合した。２つの群へのペプチドの結合の差は、統計的に高度に有意であった（スチューデントｔ検定を使用して、1.1×10^{-11}）。

【実施例３】

【００３１】

認められている精神医学的解析と比較した本発明のペプチドに基づく精神分裂病の解析
上記実施例２に記載の結果のさらなる解析により、患者の他の記録された精神医学的パラメータについて、精神分裂病の持続期間と、光学密度（ＯＤ）として測定した本発明のペプチドへの結合レベルの間に逆相関が見られた。係数ａ＝１．９３７６とｂ＝０．０００７９８を用いて幾何学的適合（$y=ax^{bx}$）を適用した（図４）。図３と４の結果を要約すると、生化学的検査を精神医学的評価と比較することにより、表１に示す以下の結果に達した。

【００３２】

表１

精神医学的評価		生化学的検査			
		罹病 1-15年	罹病 16-30年	罹病 1-30年	対照
試験者の数	N=39	N=23	N=16	N=39	N=50
精神分裂病	39	19	7	26	0
境界	0	1	6	7	6
陰性	0	3	3	6	44

これは、以下の定義を考慮する：
陰性：０から平均＋１ｓまで
境界：平均＋１ｓから平均＋２ｓまで
陽性：平均＋２ｓより大きい

【００３３】

結論として、８０％より大きい感度と９０％より大きい特異性が適しているようである。しかし、これらの定義は任意であり、従って特異性を重視するかまたは感度を重視するかにより変化することに注意されたい。

【図面の簡単な説明】

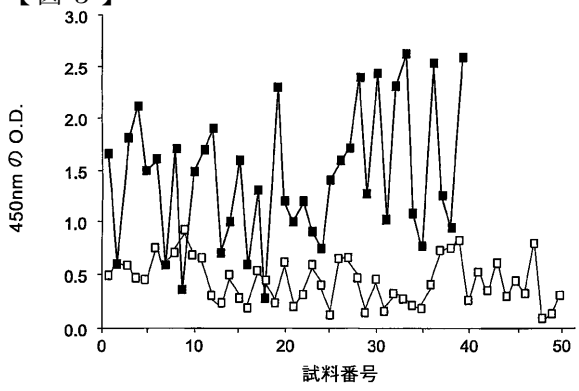
【００３４】

【図１】先行技術のペプチドは自己抗体の天然の結合部位ではあり得ないことを示す、エノラーゼの表面を示すグラフ。明らかなように、アルギニンＲ４０２（矢印）のみが結合抗体にとって利用可能であり、ペプチドの残りの部分はタンパク質内に埋まっている。
【図２】４つの陽性荷電アミノ酸（Ｒ４１４、Ｒ４０２、Ｒ１８４およびＫ１９４）に囲まれた６つの中性アミノ酸（Ｌ４１３、Ｌ１８３、Ｌ４０９、Ｌ４０６、Ｐ４００およびＡ４０１）からなる、本発明のペプチドの推定エピトープを示すグラフ。
【図３】酵素免疫定量法でビオチン化ペプチドＬＶＶＧＬＣＫを用いて測定した、３９人の精神分裂病患者（黒四角）と５０人の精神分裂者（白四角）からの血漿試料の結合レベ

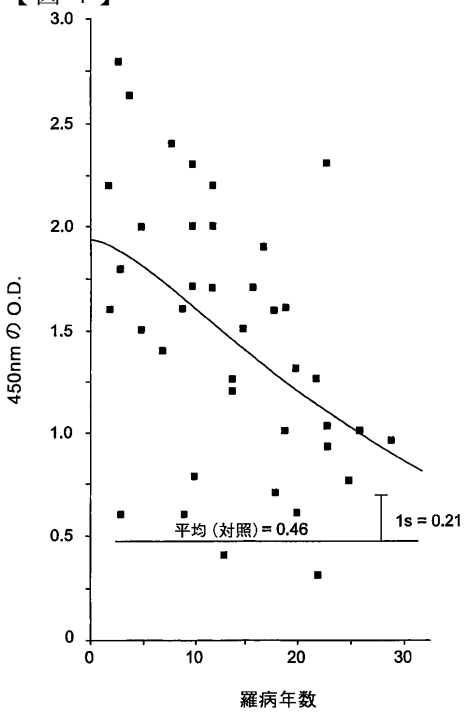
ルを示す略図。

【図４】精神分裂病患者から得られた試料中の上記酵素免疫定量法で測定したＯＤ値と、これらの患者における疾患の持続期間との逆相関を示す略図。

【図３】



【図４】



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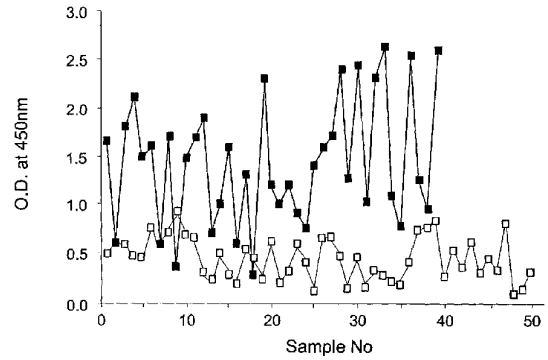
(54) Title: NOVEL PEPTIDES FOR THE DIAGNOSIS OF SCHIZOPHRENIA



(57) Abstract: Short peptides are provided, which bind to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than to a body fluid sample obtained from a non-schizophrenic individual. The peptides are no more than 10 amino acids long and comprise a continuous sequence of at least 5 amino acids which consists of at least one positively charged amino acid at one of its ends. The provided peptides, which are the putative binding sites of autoantibodies found in high levels in schizophrenic individuals, are thus useful in diagnosis of schizophrenia.

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NOVEL PEPTIDES FOR THE DIAGNOSIS OF SCHIZOPHRENIA

FIELD OF THE INVENTION

The present invention concerns an assay for the diagnosis of schizophrenia and provides novel peptides for use in the assay.

PRIOR ART

The following is a list of publications intended for better understanding of the Background of the Invention. The acknowledgement herein of the above prior art should not be construed as an indication that this art is in any way relevant to the patentability of the invention as defined in the appended claims.

Carpenter, W.T., and Buchanan, R.W.: Review, N. England., *J. Med.*, **330**:681-690, 1994.

Deckmann, M., Shinitzky, M., Leykin, I., Cheng, D., Guy, J., Avnon, M., Salganik, I., Amiri, Z., Schlossberg, A., Leibu, E., and Rafael, C.: *The Italian J. Psychiatr. Behav. Sci.*, **6**:29-34, 1996.

DeLisi, L.E. and Crow, T.J.: *Psychiatr. North Am.*, **9**:115-132, 1987.

Rotman, A.: Blood platelets in psychopharmacological research. *Prog. Neuropsychopharmacol.*, **6**:135-151, 1983.

Shinitzky, M., Deckmann, M., Kessler, A., Sirota, P., Rabbs, A., and Elizur, A.: *Ann. N.Y. Acad. Sci.*, **621**:205-217, 1991.

Shinitzky, M., and Deckmann, M., "Diagnosis of the susceptibility of contracting schizophrenia", US Patent No. 6,008,001, 1999.

Shinitzky, M., and Deckmann, M., "Assay for schizophrenia based on skin reaction" WO 99/30163, 1999.

Shinitzky, M., and Deckmann, M., "Assay for the diagnosis of schizophrenia based on a new peptide" WO 99/51725, 1999.

BACKGROUND OF THE INVENTION

Schizophrenia is a syndrome which encompasses a variety of mental symptoms like auditory hallucinations, paranoia, delusions, catatonia, bizarre behavior and emotional withdrawal. Schizophrenia affects about 1% of the total population and its economical as well as social burden on society are enormous. The onset of the disease occurs in early age and, thus, patients typically need life-long medical and psychiatric supervision. Schizophrenia is, therefore, rated as one of the most costly diseases in the industrial world (Carpenter, *et al.,* 1994).

No common parameter associated with schizophrenia has been identified and, therefore, the internationally agreed diagnosis of this disease is still based today solely on psychiatric evaluation. Known risk factors associated with schizophrenia, are genetic predisposition, birth during winter and complications during pregnancy or birth. Viral and/or bacterial infections with a subsequent autoimmune reaction have been proposed as causative factors for the increasing outbreak of schizophrenia (DeLisi, *et al.*, 1987).

Schizophrenia has been shown to involve an autoimmune process and lately autoantibodies and cytotoxic T-cells against platelets were demonstrated in schizophrenic patients (Shinitzky, 1991, Deckmann, 1996, Shinitzky, 1999, US Patent 6,008,001). The cytotoxic T-cell reaction in schizophrenic patients was evaluated by a skin test in which most schizophrenic patients reacted positively against their autologous platelets whereas only a very minor number of non-schizophrenic tested individuals reacted positively in this test (Shinitzky, 1999, WO99/30163).

In addition elevated levels of autoantibodies against platelets were observed in schizophrenic patients but not in patients suffering from manic-depressive disorder, depression, personality disorders and schizoaffective disorder (Shinitzky, 1991 and Deckmann, 1996).

In the inventors' prior work, several proteins which bind autoantibodies that are found in elevated levels in body fluids of schizophrenic patients were identified (Shinitzky *et al.*, 1999, WO 99/51725). These proteins reacted with purified platelet

derived autoantibodies (PAA) from schizophrenic patients but could not differentiate between plasma or blood samples of schizophrenic and non-schizophrenic individuals. Enzymatic digestion of one of these proteins, the enzyme Enolase, resulted in a fragment which bound to plasma samples of schizophrenic patients substantially higher than it bound to plasma samples of non-schizophrenic individuals. On the basis of this fragment several additional peptides were synthesized and such having a high binding activity to PAAs of schizophrenic individuals were isolated. The structure of the antigenic epitope of these peptides was found to be a three-dimensional epitope which, by using a computerized program was predicted to be a cyclic structure comprising a hydrophobic core and an extension having about two positive charges. Immunological studies demonstrated that only the oxidized cyclic form of the peptide was reactive with the anti-platelet autoantibodies. These synthesized peptides comprised at least 17 amino acids (a.a.).

SUMMARY OF THE INVENTION

In accordance with the present invention, it has been realized that the peptide sequences described in the prior art (WO 99/51725) are able to bind to a higher extent to autoantibodies which are found in elevated levels in body fluids of schizophrenic patients and to a lower extent or not at all in body fluids of non-schizophrenic individuals, but these peptides cannot be the natural binding site for such autoantibodies. This is due to the fact that the sequences are not exposed on the surface of the protein from which they were derived (the enzyme enolase). Except for the single amino acid arginine at position 402, the remaining amino acids are buried inside the protein (see Fig. 1). In addition, as known, an antibody binding site is usually comprised of about 5-8 a.a. while each of these peptides comprised 17 a.a. which are not all necessary for the binding site.

Therefore, in accordance with the present invention, based on the realization that the peptide which is the natural binding site for autoantibodies found in elevated levels in schizophrenic patients will have a more sensitive and specific

binding to such autoantibodies and as such may be advantageous in diagnosis of schizophrenia, an attempt was made to identify an equivalent site on the surface of the enolase by using three-dimensional modeling. Using the three-dimensional structure to search the surface of the enolase resulted in the identification of a putative epitope (see Fig. 2) consisting of four positively charged amino acids defined as R414, R184, K194 and R402 which surround a cluster of neutral amino acids defined as L412, L183, L409, L406, P400 and A401. Peptides having such a putative epitope are provided in accordance with the invention.

Thus, by its first aspect, the present invention provides a peptide which binds to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than its binding to a body fluid sample obtained from a non-schizophrenic individual, said peptide being no more than 10 amino acids (a.a.) long and comprising a continuous sequence of at least 5 amino acids included in any one of the following sequences :

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4);

said continuous sequence consisting of at least one positively charged a.a. at one end of said sequence; and said peptide comprising at least one positively charged a.a at its end being the positively charged a.a of said continuous sequence or at least one additional positively charged a.a.;

or analogues of said peptide being no more than 10 a.a long and in which no more than two a.a of said continuous sequence are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptide.

A *"substantively higher level of binding"* in accordance with the invention will be determined by using any of the binding assays known in the art such as those described below and wherein the measured level of binding of a peptide to a sample obtained from a schizophrenic patient is significantly higher than the measured level of binding of the same peptide to a sample obtained from a

non-schizophrenic patient as determined by a suitable statistic test, e.g. Student's T-test.

The term "*continuous sequence*" concerns an uninterrupted sequence of between 5 and 7 a.a of any of the sequences of SEQ.IDs 1-4 which includes a positively charged a.a at its end. The positively charged a.a is preferably Lysine (indicated as K in the sequences) but may also be Arginine (R) or Histidine (H).

The continuous sequence can be part of a longer peptide of up to 10 a.a., wherein the continuous sequence is situated anywhere in the peptide. In case the peptide consists of more than 7 a.a, wherein the continuous sequence is at one of the peptides ends, said positively charged a.a will be at the open end of the sequence (which is not connected to the additional a.a of the longer peptide) so that the large peptide comprises a positively charged a.a at one of its ends . Wherein the continuous sequence is in the middle of the peptide, the peptide comprises at least one additional positively charged a.a. at one of its ends in addition to the positively charged a.a of the continuous sequence.

Analogues of the above peptides are also within the scope of the present invention. Such analogues are peptides which comprise no more than 10 a.a including at least 5 a.a which have the same sequence as one of the above mentioned continuous sequences but in which one or two a.a are conservatively replaced, as this term is defined below. The analogues also comprise at least one positively charged a.a at their end and essentially maintain the activity of the peptides as this term is defined below.

The term *"essentially maintains the binding characteristics"* refers to a peptide which level of binding to the tested sample is at least 50%, preferably 70%, most preferably 90% or more than 100% of the level of binding of the peptide to the same tested sample as determined by the same binding assay.

By a preferred embodiment, the invention provides a peptide which binds to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than it binds to a body fluid sample obtained from a non-schizophrenic individual said peptide selected from the group consisting of:

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4)

or analogs of said peptides in which no more than two a.a are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptides.

By a most preferred embodiment, a peptide is provided which binds to body fluid samples obtained from a schizophrenic patient substantively higher than it binds to a body fluid sample obtained from a non-schizophrenic individual having the amino acid sequence LVVGLCK (SEQ ID NO. 1).

By an additional aspect of the invention, a peptide is provided which binds to a body sample obtained from a schizophrenic patient substantively higher than it binds to a sample obtained from a non-schizophrenic individual, wherein the peptide binds antibodies that are capable of specific binding to a peptide having the amino acid LVVGLCK. Several non limiting examples of such peptides are the following:

i. KLVVGLC (SEQ ID NO. 2)
ii. LVVGLMK (SEQ ID NO. 3)
iii. KLVVGLM (SEQ ID NO. 4)

or analogs thereof in which no more than two a.a are conservatively replaced and which maintain the binding characteristics of the peptides.

The letters used above and throughout the present description to denote specific amino acids (a.a.) are in accordance with the one letter a.a. symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

In view of the fact that the peptides of the invention are the putative natural binding sites for autoantibodies found in elevated levels in body fluids of schizophrenic patients, and due to their high purity and high activity, these peptides are most useful for the diagnosis of schizophrenia. Thus, by an additional aspect,

the present invention provides an assay for the diagnosis of schizophrenia in an individual comprising the following steps:

(a) obtaining a body fluid sample from said individual being a blood sample, platelet-associated antibodies (PAA) containing fraction thereof, or a fraction containing PAA shed from the platelets;

(b) contacting said sample with a peptide being no more than 10 amino acids (a.a.) long and comprising a continuous sequence of at least 5 amino acids included in any one of the following sequences :

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4);

said continuous sequence consisting of at least one positively charged a.a. at one end of said sequence; and said peptide comprising at least one positively charged a.a at its end being the positively charged a.a of said continuous sequence or at least one additional positively charged a.a.;

or analogs of said peptide being no more than 10 a.a long and in which no more than two a.a of said continuous sequence are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptide.

(c) determining the level of binding of said peptide to said sample, a level of binding substantively higher than the level of binding of said peptide to a sample obtained from a non-schizophrenic individual indicating that said tested individual has a high likelihood of having schizophrenia.

By a preferred embodiment, the peptide of step (b) is selected from the group consisting of:

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4)

or analogs thereof in which no more than two a.a are conservatively replaced and which essentially maintain said peptide's binding characteristics.

By a preferred embodiment, the peptide of step (b) has the amino acid sequence LVVGLCK.

By an additional aspect, the peptides in step (b) are such which bind antibodies which bind peptides having the amino acid sequence LVVGLCK or analogues thereof.

Use of the peptides of the invention and analogues thereof as defined above and below for the preparation of a diagnostic composition for diagnosis of schizophrenia in an individual is also within the scope of the present invention.

By an additional aspect, the invention provides a kit useful in the above assay, said kit comprising a support comprising one or more peptides of the invention immobilized onto it, an anti-human immunoglobulin (hIg) antibody or fragment thereof, or one or more non-based peptides conjugated to a detectable marker which bind to antibodies present in the tested sample, reagents required for carrying out the detection assay wherein said peptides bind to antibodies present in a tested sample as well as instructions for use.

Wherein the detection of the binding of the peptides of the invention to the tested sample is by an anti-hIg antibody the anti-hIg antibody may be conjugated to a detectable marker or alternatively, the kit may also comprise a second type of antibodies directed against said first antibodies, wherein the second antibodies are conjugated to a detectable marker.

By one embodiment, the binding of the peptide of the invention to the tested sample is detected using second non-bound peptides complexed with a detectable marker, said second peptides capable of binding to the antibodies present in the tested sample. In accordance with this embodiment, the detection is achieved by a double antigen sandwich text which may be performed as a one step assay or as a two step assay. Wherein the detection is performed by the double antigen sandwich text assay, the kit of the invention will include such

peptides conjugated to a detectable marker instead of the anti-human immunoglobulin antibody.

The assay of the invention may be used as a single test for detecting a high likelihood of schizophrenia in an individual. However, in accordance with an additional aspect of the Invention, the peptides and assay may be used as a confirmatory diagnostic tool. Thus, for example, wherein a high likelihood of schizophrenia is determined in an individual by methods used to date (mainly psychiatric evaluation, as mentioned above), this could be reaffirmed (or, alternatively, re-evaluated) by using the assay of the Invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphical representation showing a surface presentation of enolase which demonstrates that the prior art peptide cannot be the natural binding site for autoantibodies. As can be seen, only arginine R402 (arrow) would be available to binding antibodies wherein the remaining part of the peptide is buried within the protein.

Fig. 2 is a graphical representation of the putative epitope of the peptides of the invention, which consists of six neutral amino acids (L413, L183, L409, L406, P400 and A401) which are surrounded by four positively charged amino acids (R414, R402, R184 and K194).

Fig. 3 is a schematic representation showing the level of binding of plasma samples from 39 schizophrenic patients (filled squares) and 50 non schizophrenic individuals (open squares) with the biotinylated peptide LVVGLCK in an enzyme immunoassay.

Fig. 4 is a schematic representation showing an inverse correlation between the O.D. values measured in the enzyme immunoassay described above in samples obtained from schizophrenic patients and the duration of the disease in these patients.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides short highly purified and highly active peptides, which comprise a putative natural epitope of the peptides to which autoantibodies found in elevated levels in schizophrenic patients, bind. The short length of these peptides (7-10 amino acids) and their structure, which is a structure, which can be exposed on the surface of the enolase enzyme in a manner, which enables the autoantibodies to bind to it, render these peptides to be most useful in the diagnosis of schizophrenia. Thus, although the peptides of the prior art (WO 99/51725) were able to bind to autoantibodies present at higher levels in schizophrenic patients as compared to non-schizophrenic individuals, the peptides of the present invention, having the above characteristics enable to detect an individual having a high probability of having schizophrenia more effectively.

The binding activity of the peptide of the invention to various antibodies may be determined by any of the methods known *per se* such as ELISA or Western Blotting. For example, a tested peptide may be analyzed for its binding activity to antibodies by subjecting it to polyacrylamide gel electrophoresis, blotting it onto PVDS membranes which are then reacted with a body fluid sample of the tested sample and compared to their reaction with a sample obtained from a non-schizophrenic individual.

The extent of binding of the peptides of the invention to PAA can be determined by using any detection system known in the art such as antibodies against human immunoglobulin or fragments thereof linked to a detectable marker. The marker may be a radioactive group, a fluorescent group, an enzyme capable of catalyzing a reaction yielding a detectable product, a biotin group capable of being detected by avidin, etc.

By a preferred embodiment, the extent of binding of the peptides of the invention to the tested sample is carried out using an enzyme immunoassay in which the peptides are labeled with biotin and bound to streptavidine coated tubes as explained below.

In accordance with the invention, it has been found that the position of the positive charge in the peptides may be either at the beginning or at the end of the peptide. This indicates that the peptides exist in an open ring form. In addition it was found that cysteine does not have importance in the sequence of the peptides since substitution of cysteine with Methionin retained the activity of the peptides. Thus, as explained above, the continuous sequence of a.a comprised within the peptide of the invention consists of a positively charged a.a at its end. If the continuous sequence is at one of the ends of the whole peptide, this positively charged a.a will be at one end of the whole peptide. Alternatively, if the continuous sequence is not at the end of the whole peptide, the whole peptide will comprise an additional positive charge at one of its ends.

It is known that the antibody binding site is usually consisted of at least five amino acids. Thus, the peptides of the Invention and their analogues comprise at least five amino acids. The five amino acids will be such which appear consecutively in one of the continuous sequences of the invention.

In order to maintain the binding characteristics of the peptide, the analogues of the invention comprise no more than two a.a. substitutions which are conservative substitutions. These are substitutions in which an amino acid of one class is replaced by an amino acid of the same class, where a class is defined by common physiochemical amino acid chain properties such as charge, size or hydrophobicity. Amino acids of the same class are characterized by high substitution frequencies in homologous proteins found in nature (as determined, for example, by a standard Dayhoff frequency exchange matrix).

Thus for example the leucine positioned in the first position of the amino acid sequence of a peptide of the invention may be conservatively substituted by the amino acids glycine or valine, which belong to the same family of amino acids, without altering the binding activity of the peptide. The positively charged a.a at the end of the peptide of the invention may be conservatively replaced by another positively charged a.a. A person versed in the art will have no difficulty in determining by which amino acid each of the amino acids of the peptide may

be replaced in accordance with the known grouping of amino acids into families as may be found, for example, in Molecular Biology of the Cell Editors Alberts B. *et al.*, Garland Publishing, Inc., New York and London, 2nd Edition, 1989, pages 54-55.

The peptides of the invention may also be chemically modified. In such a chemically modified peptide at least one of the amino acid residues may be modified either by a natural process such as post-translational modification or by chemical modification techniques which are well known in the art. Examples of chemical modifications are acetylations and glycolysations, glycosamine-glycinations, ADP-ribosylations, covalent attachment of a lipid or a lipid derivative, methylation, myristylation, pegylation, phosphorylation, etc. The chemical modification may be at the peptide's amino end or at the carboxy end.

The peptides of the invention may additionally have a non peptide component attached such as for example a macromolecular carrier group which may covalently be attached to the amino or carboxy side of the peptide. Such a carrier may for example be polyethylene glycol, carbohydrates or lipid fatty acid conjugates.

All the above describe changes in the peptides of the invention that may result in increased stability, bioavailability or activity of the peptides of the invention.

The peptide of the invention may be obtained by enzymatic digestion (e.g. using Clostrapain) or chemical (CNBr) digestion of a longer protein. In such a case, the resulting peptides are separated by methods known in the art such as by RP-HPLC and the separate peptides may then be used for sequencing (e.g. by Eurosequence b.v. (Nijenborgh 4; 9749 Gronigen; The Netherlands)) and analyzed for their binding capability to antibodies as described above.

The short length of the peptides of the Invention (5 to 10 a.a) renders them as very good candidates for synthesize by methods known in the art such as on Abimed 522 at a 10 μmol scale by Eurosequence b.v. (see detailed explanation in

the examples below). The binding activity of the newly synthesized peptides will be determined using any of the assays mentioned above.

Another advantages of the present invention, which is based on the fact that the peptides of the invention are short, purified and active peptides, is that the *"body fluid"* sample of the individual to be tested may be a blood plasma or serum sample which is relatively easy to obtain and prepare. However, at times it may be advantageous to perform the assay of the invention on a blood sample obtained from the tested individual which is a PAA containing fraction obtained from the sample by any of the methods known in the art, such as by obtaining a platelet-rich plasma and isolating PAA therefrom. In accordance with the invention, the sample may also be any other body fluid sample obtained from the tested individual including a whole blood sample, or any other body fluid sample containing PAA, e.g. saliva, cerebrospinal fluid, etc. Chemical analysis as well as immunoactivity assays in accordance with the invention showed that the peptide having the amino acid sequence LVVGLCK had the highest purity of synthesis and could spontaneously form dimers thus doubling the epitope per binding site of streptavidine and enhancing the binding potential of PAAs to this peptide. Therefore, in accordance with the invention, a peptide having the above sequence or a peptide comprising this sequence are preferred for use in the diagnosis of schizophrenia in accordance with the invention.

EXAMPLES

The invention will now be demonstrated by the following non limiting Examples with reference to the figures.

Materials and Methods

1. Patients and control persons – Thirty-nine schizophrenic patients participated in this study. The following parameters were recorded: gender, age, duration of schizophrenia, number of hospitalizations, years of

education and the psychiatric status (PANSS). Fifty plasma samples were obtained from a local blood bank.

2. Plasma – Venous blood was drawn with heparin as coagulant from patients and control subjects. Plasma was obtained after centrifugation (4000 g; 15 mins. 4°C).

3. Peptide synthesis and biotin labeling – The peptides were synthesized on an Abimed 522 at a 10 micromol scale by Eurosequence b.v., (Nijenborgh 4: 9747 AG Groningen; The Netherlands) and labeled at the amino terminal with biotin containing a six carbon spacer. Peptide purity was routinely assessed by RP-HPLC and, when considered necessary, by laser desorption mass spectroscopy. The peptides were routinely dissolved in 1 ml water/DMF/DMSO (1:1:1; v/v/v).

4. Line blot – peptides were line blotted onto PVDF or nitrocellulose membranes. Membrane strips were incubated overnight with 0.5 ml plasma sample in 0.5 ml buffer (200 mM Tris; 0.3% Casein; 200 mM KCl; 10,6 mM phenol; 2.1 mM $CaCl_2$; 0.01% Triton X-100; pH 8.5) at room temperature under gentle shaking in BioRad incubation arrays. After three washings with PBS, strips were probed with Anti-human Fc (goat) conjugated to horseradish peroxidase (SIGMA; dilution 1:100) for 2 hours at room temperature under gentle shaking. Fast-DAB™ or 4-Chloro- naphthol (SIGMA) were used as color reagent to detect bound antibodies.

5. Enzyme immuno assay – Streptavidine coated tubes (Boehringer Mannheim; 80 nMol Streptavidine per tube) were coated with a tenfold excess of biotin-labeled peptide in 1 ml PBS for 3 days at 4°C, washed twice with 2 ml 1M NaCl, once with 2 ml water, dried and stored under vacuum at minus 18°C until use. For the test, 0.05 ml sample and 1 ml Incubation

Buffer (60 mM citric acid; 90 mM Na_2HPO_4; 168 mM NaOH; 200 mM NaCl; pH 7.7) are added to the peptide labeled tube and incubated for 1 hr at 37°C without shaking. The tubes were covered in order to prevent evaporation. The tubes were then washed three times with 2 ml 1 M NaCl solution for 15 min. under gentle shaking, 1 ml Anti-human Fc (goat) conjugated to horseradish peroxidase (SIGMA) added at a dilution of 1:2000 in POD Buffer (200 mM Tris; 0.3% Casein; 200 mM KCl; 10,6 mM phenol; 2.1 mM $CaCl_2$; 0.01% Triton X-100; pH 8.0) and incubated for 0.5 hr at 37°C without shaking. After 4 washings with 2 ml 1M NaCl solution, the color reaction was initiated by adding 1 ml Tetramethylbenzidine (TMB) Liquid Substrate System™ (SIGMA). The enzymatic reaction was stopped by adding 1 ml 0.5M H_2SO_4. The absorbence was read at 450 nm spectrophotometrically.

Example 1:

Four peptides in accordance with the invention, having one of the a.a. sequences:

(a) LVVGLCK

(b) KLVVGLC

(c) LVVGLMK

(d) KLVVGLM

were biotin-labeled and coated onto streptavidine coated tubes as described above. A pool of plasma samples was prepared from 5 schizophrenic patients and an additional pool of plasma was prepared from 5 non-schizophrenic individuals.

Results:

All of the four peptides of the invention bound to the plasma pool originating from schizophrenic patients to a higher extent than their binding to the control plasma sample obtained from non-schizophrenic individuals (as measured in the enzyme immunoassay described above which showed 0.5 O.D. in the

samples of the peptides with the control plasma samples and 1.6 O.D. in the samples of the peptides with the schizophrenia derived plasma pool).

Example 2: Diagnosis of schizophrenia using the peptide LVVGLCK

Of the four peptides of the invention, the highest purity of synthesis was found in the peptide LVVGLCK. In addition, these peptides were found to spontaneously form dimers, thus doubling the epitope for binding site of streptavidine.

Therefore, an experiment was carried out with the biotin-LVVGLCK as follows: the peptide was biotin-labeled and coated onto streptavidine coated tubes as explained above. Plasma samples of 39 schizophrenic patients and 50 control non schizophrenic individuals were tested with the coated peptide and the level of binding was determined by the enzyme immunoassay described above.

As seen in Fig. 3, the biotinylated peptide LVVGLCK bound to a higher extent to the plasma samples obtained from schizophrenic patients (filled squares) at a mean value and standard deviation of 1.47 ± 0.65 as compared to its binding to plasma samples from non schizophrenic individuals (open squares) with mean value and standard deviation amounting to 0.46 ± 0.21. The difference of binding of this peptide to the two groups was statistically highly significant (1.1×10^{-11} using Student's T-test).

Example 3: Analysis of schizophrenia on the basis of the peptide of the invention as compared to acceptable psychiatric analysis

By further analysis of the results described in Example 2 above, with other recorded psychiatric parameters of the patients, an inverse correlation was found between the duration of schizophrenia and the level of binding to the peptide of the invention measured as optical density (O.D.). A geometric fit ($y = ax^{bx}$) was applied with $a = 1.9376$ and $b = 0.000798$ as coefficients (Fig. 4). Summarizing Figs. 3 and 4, the following results shown in Table 1 below can be reached by comparing the biochemical test with the psychiatric evaluation.

Table 1

Psychiatric Evaluation		**Biochemical Test**			
		1-15 years disease	16-30 years disease	1-30 years disease	Controls
No. of tested individuals	N = 39	N = 23	N = 16	N = 39	N = 50
Schizophrenic:	39	19	7	26	0
Borderline:	0	1	6	7	6
Negative	0	3	3	6	44

This takes the following definitions into account:

Negative: 0 until mean + 1s

Borderline: Between mean + 1s until mean + 2s

Positive: Higher than mean + 2s

In conclusion, a sensitivity of better than 80% and a specificity of better than 90% seem to be feasible. However, it should be noted that these definitions are arbitrary and may, therefore, be changed depending whether the emphasis is more on specificity or on sensitivity.

CLAIMS:

1. A peptide which binds to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than its binding to a body fluid sample obtained from a non-schizophrenic individual, said peptide being no more than 10 amino acids (a.a.) long and comprising a continuous sequence of at least 5 amino acids included in any one of the following sequences :

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4);

said continuous sequence consisting of at least one positively charged a.a. at one end of said sequence; and said peptide comprising at least one positively charged a.a at its end being the positively charged a.a of said continuous sequence or at least one additional positively charged a.a.;

or analogs of said peptides being no more than 10 a.a long and in which no more than two a.a of said continuous sequence are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptides.

2. A peptide which binds to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than its binds to a body fluid sample obtained from a non-schizophrenic individual said peptide selected from the group consisting of:

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4)

or analogs of said peptides in which no more than two a.a are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptides.

3. A peptide which binds to body fluid samples obtained from a schizophrenic patient substantively higher than it binds to a body fluid sample obtained from a

non-schizophrenic individual having the amino acid sequence LVVGLCK (SEQ ID NO. 1).

4. A peptide which binds to a body sample obtained from a schizophrenic patient substantively higher than it binds to a sample obtained from a non-schizophrenic individual, wherein the peptide binds antibodies that are capable of specific binding to a peptide having the amino acid LVVGLCK (SEQ ID NO.1).

5. A peptide according to Claim 4, having the amino acid sequence KLVVGLC (SEQ ID NO. 2).

6. A peptide according to Claim 4, having the amino acid sequence LVVGLMK (SEQ ID NO. 3).

7. A peptide according to Claim 4, having the amino acid sequence KLVVGLM (SEQ ID NO. 4).

8. An assay for the diagnosis of schizophrenia in an individual comprising the following steps:

(a) obtaining a body fluid sample from said individual being a blood sample, PAA-containing fraction thereof, or a fraction containing platelet-associated antibodies (PAA) shed from the platelets;

(b) contacting said sample with a peptide being no more than 10 amino acids (a.a.) long and comprising a continuous sequence of at least 5 amino acids included in any one of the following sequences :

i. LVVGLCK (SEQ ID NO. 1)

ii. KLVVGLC (SEQ ID NO. 2)

iii. LVVGLMK (SEQ ID NO. 3)

iv. KLVVGLM (SEQ ID NO. 4);

said continuous sequence consisting of at least one positively charged a.a. at one end of said sequence; and said peptide comprising at least one positively charged a.a at its end being the positively charged a.a of said continuous sequence or at least one additional positively charged a.a.;

or analogs of said peptides being no more than 10 a.a long and in which no more than two a.a of said continuous sequence are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptides.

(c) determining the level of binding of said peptide to said sample, a level substantively higher than the binding level of said peptide to a sample obtained from a non- schizophrenic individual indicating that said tested individual has a high likelihood of having schizophrenia.

9. A method according to Claim 8, wherein the peptide of step (b) is selected from the group consisting of:

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4)

or analogs thereof in which no more than two a.a are conservatively substituted and which essentially maintain said peptides' binding characteristics.

10. A method according to Claim 8, wherein the peptide of step (b) has the amino acid sequence LVVGLCK (SEQ ID NO.1).

11. A method according to Claim 8, wherein the peptide in step (b) is such which binds antibodies which bind peptides having the amino acid sequence LVVGLCK (SEQ ID NO. 1) or analogs thereof in which no more than two a.a. are conservatively substituted.

12. A kit for use in the diagnosis of schizophrenia comprising a support comprising one or more peptides of Claim 1, immobilized onto it, anti-human immunoglobulin (hIg) antibody or fragment thereof, reagents carrying a detection assay whereby said peptides bind to antibodies present in a tested sample, as well as instructions for use.

13. A kit according to Claim 12, wherein said anti-hIg antibody is complexed to a detectable marker.

14. A kit according to Claim 12, wherein instead of said anti-hIg antibody, the kit comprises one or more non-bound peptides which bind to antibodies present in a tested sample, said peptides complexed to a detectable marker.

15. Use of any of the peptides of Claim 1 for the preparation of a diagnostic composition for the diagnosis of schizophrenia in an individual.

16. The assay of Claim 8, for use in confirming a high probability of Schizophrenia in an individual determined by at least one other diagnostic assay.

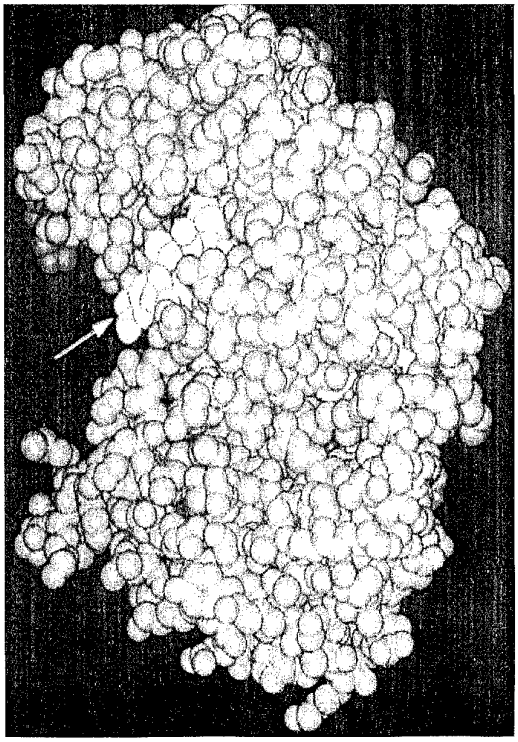

FIG.1

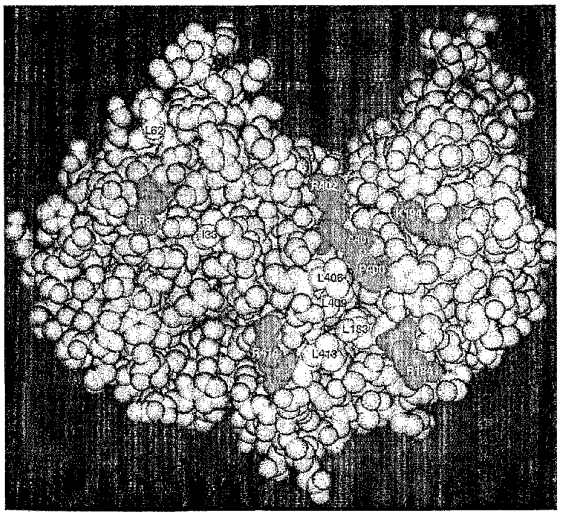

FIG.2

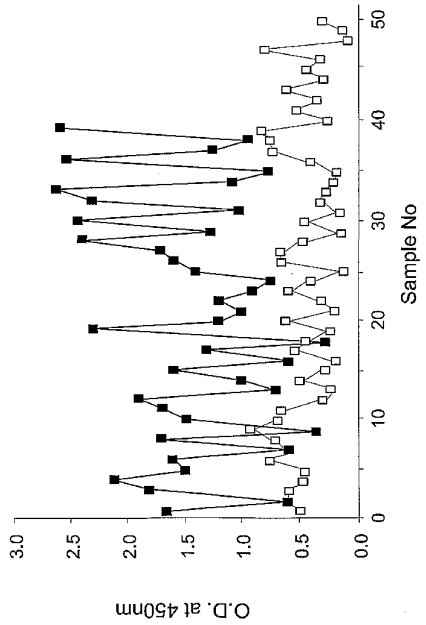

FIG. 3

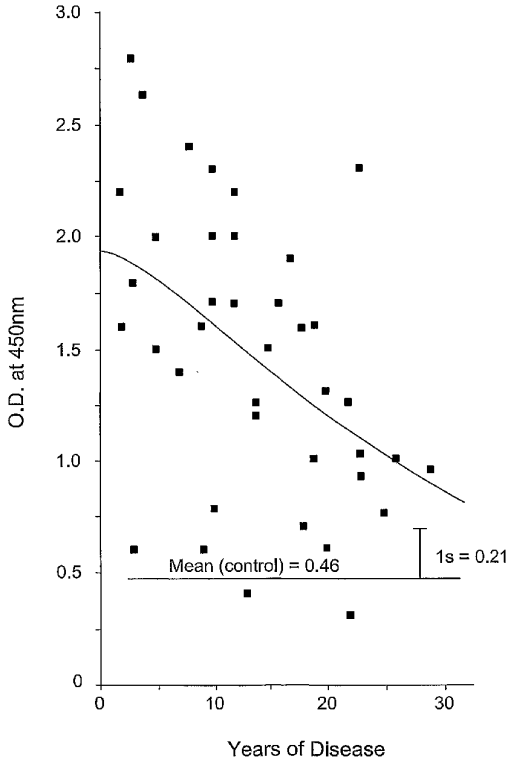
3.0
2.5
2.0
1.5
1.0
0.5
0
O.D. at 450nm
0
10
20
30
Years of Disease
Mean (control) = 0.46
1s = 0.21

FIG. 4

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(54) Title: NOVEL PEPTIDES FOR THE DIAGNOSIS OF SCHIZOPHRENIA

(57) Abstract: Short peptides are provided, which bind to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than to a body fluid sample obtained from a non-schizophrenic individual. The peptides are no more than 10 amino acids long and comprise a continuous sequence of at least 5 amino acids which consists of at least one positively charged amino acid at one of its ends. The provided peptides, which are the putative binding sites of autoantibodies found in high levels in schizophrenic individuals, are thus useful in diagnosis of schizophrenia.

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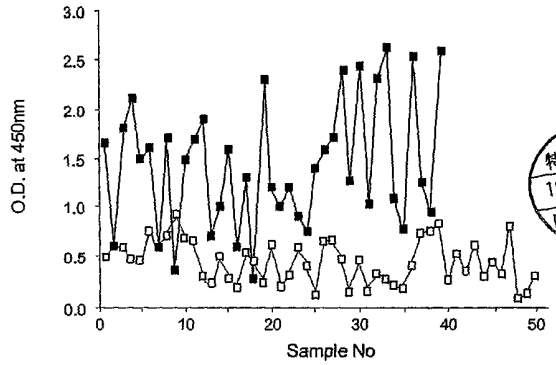
(54) Title: NOVEL PEPTIDES FOR THE DIAGNOSIS OF SCHIZOPHRENIA



(57) Abstract: Short peptides are provided, which bind to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than to a body fluid sample obtained from a non-schizophrenic individual. The peptides are no more than 10 amino acids long and comprise a continuous sequence of at least 5 amino acids which consists of at least one positively charged amino acid at one of its ends. The provided peptides, which are the putative binding sites of autoantibodies found in high levels in schizophrenic individuals, are thus useful in diagnosis of schizophrenia.

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NOVEL PEPTIDES FOR THE DIAGNOSIS OF SCHIZOPHRENIA

FIELD OF THE INVENTION

The present invention concerns an assay for the diagnosis of schizophrenia and provides novel peptides for use in the assay.

PRIOR ART

The following is a list of publications intended for better understanding of the Background of the Invention. The acknowledgement herein of the above prior art should not be construed as an indication that this art is in any way relevant to the patentability of the invention as defined in the appended claims.

Carpenter, W.T., and Buchanan, R.W.: Review, N. England., *J. Med.*, 330:681-690, 1994.

Deckmann, M., Shinitzky, M., Leykin, I., Cheng, D., Guy, J., Avnon, M., Salganik, I., Amiri, Z., Schlossberg, A., Leibu, E., and Rafael, C.: *The Italian J. Psychiatr. Behav. Sci.*, 6:29-34, 1996.

DeLisi, L.E. and Crow, T.J.: *Psychiatr. North Am.*, 9:115-132, 1987.

Rotman, A.: Blood platelets in psychopharmacological research. *Prog. Neuropsychopharmacol.*, 6:135-151, 1983.

Shinitzky, M., Deckmann, M., Kessler, A., Sirota, P., Rabbs, A., and Elizur, A.: *Ann. N.Y. Acad. Sci.*, 621:205-217, 1991.

Shinitzky, M., and Deckmann, M., "Diagnosis of the susceptibility of contracting schizophrenia", US Patent No. 6,008,001, 1999.

Shinitzky, M., and Deckmann, M., "Assay for schizophrenia based on skin reaction" WO 99/30163, 1999.

Shinitzky, M., and Deckmann, M., "Assay for the diagnosis of schizophrenia based on a new peptide" WO 99/51725, 1999.

BACKGROUND OF THE INVENTION

Schizophrenia is a syndrome which encompasses a variety of mental symptoms like auditory hallucinations, paranoia, delusions, catatonia, bizarre behavior and emotional withdrawal. Schizophrenia affects about 1% of the total population and its economical as well as social burden on society are enormous. The onset of the disease occurs in early age and, thus, patients typically need life-long medical and psychiatric supervision. Schizophrenia is, therefore, rated as one of the most costly diseases in the industrial world (Carpenter, *et al.,* 1994).

No common parameter associated with schizophrenia has been identified and, therefore, the internationally agreed diagnosis of this disease is still based today solely on psychiatric evaluation. Known risk factors associated with schizophrenia, are genetic predisposition, birth during winter and complications during pregnancy or birth. Viral and/or bacterial infections with a subsequent autoimmune reaction have been proposed as causative factors for the increasing outbreak of schizophrenia (DeLisi, *et al.*, 1987).

Schizophrenia has been shown to involve an autoimmune process and lately autoantibodies and cytotoxic T-cells against platelets were demonstrated in schizophrenic patients (Shinitzky, 1991, Deckmann, 1996, Shinitzky, 1999, US Patent 6,008,001). The cytotoxic T-cell reaction in schizophrenic patients was evaluated by a skin test in which most schizophrenic patients reacted positively against their autologous platelets whereas only a very minor number of non-schizophrenic tested individuals reacted positively in this test (Shinitzky, 1999, WO99/30163).

In addition elevated levels of autoantibodies against platelets were observed in schizophrenic patients but not in patients suffering from manic-depressive disorder, depression, personality disorders and schizoaffective disorder (Shinitzky, 1991 and Deckmann, 1996).

In the inventors' prior work, several proteins which bind autoantibodies that are found in elevated levels in body fluids of schizophrenic patients were identified (Shinitzky *et al.*, 1999, WO 99/51725). These proteins reacted with purified platelet

derived autoantibodies (PAA) from schizophrenic patients but could not differentiate between plasma or blood samples of schizophrenic and non-schizophrenic individuals. Enzymatic digestion of one of these proteins, the enzyme Enolase, resulted in a fragment which bound to plasma samples of schizophrenic patients substantially higher than it bound to plasma samples of non-schizophrenic individuals. On the basis of this fragment several additional peptides were synthesized and such having a high binding activity to PAAs of schizophrenic individuals were isolated. The structure of the antigenic epitope of these peptides was found to be a three-dimensional epitope which, by using a computerized program was predicted to be a cyclic structure comprising a hydrophobic core and an extension having about two positive charges. Immunological studies demonstrated that only the oxidized cyclic form of the peptide was reactive with the anti-platelet autoantibodies. These synthesized peptides comprised at least 17 amino acids (a.a.).

SUMMARY OF THE INVENTION

In accordance with the present invention, it has been realized that the peptide sequences described in the prior art (WO 99/51725) are able to bind to a higher extent to autoantibodies which are found in elevated levels in body fluids of schizophrenic patients and to a lower extent or not at all in body fluids of non-schizophrenic individuals, but these peptides cannot be the natural binding site for such autoantibodies. This is due to the fact that the sequences are not exposed on the surface of the protein from which they were derived (the enzyme enolase). Except for the single amino acid arginine at position 402, the remaining amino acids are buried inside the protein (see Fig. 1). In addition, as known, an antibody binding site is usually comprised of about 5-8 a.a. while each of these peptides comprised 17 a.a. which are not all necessary for the binding site.

Therefore, in accordance with the present invention, based on the realization that the peptide which is the natural binding site for autoantibodies found in elevated levels in schizophrenic patients will have a more sensitive and specific

binding to such autoantibodies and as such may be advantageous in diagnosis of schizophrenia, an attempt was made to identify an equivalent site on the surface of the enolase by using three-dimensional modeling. Using the three-dimensional structure to search the surface of the enolase resulted in the identification of a putative epitope (see Fig. 2) consisting of four positively charged amino acids defined as R414, R184, K194 and R402 which surround a cluster of neutral amino acids defined as L412, L183, L409, L406, P400 and A401. Peptides having such a putative epitope are provided in accordance with the invention.

Thus, by its first aspect, the present invention provides a peptide which binds to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than its binding to a body fluid sample obtained from a non-schizophrenic individual, said peptide being no more than 10 amino acids (a.a.) long and comprising a continuous sequence of at least 5 amino acids included in any one of the following sequences :

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4);

said continuous sequence consisting of at least one positively charged a.a. at one end of said sequence; and said peptide comprising at least one positively charged a.a at its end being the positively charged a.a of said continuous sequence or at least one additional positively charged a.a.;

or analogues of said peptide being no more than 10 a.a long and in which no more than two a.a of said continuous sequence are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptide.

A *"substantively higher level of binding"* in accordance with the invention will be determined by using any of the binding assays known in the art such as those described below and wherein the measured level of binding of a peptide to a sample obtained from a schizophrenic patient is significantly higher than the measured level of binding of the same peptide to a sample obtained from a

non-schizophrenic patient as determined by a suitable statistic test, e.g. Student's T-test.

The term *"continuous sequence"* concerns an uninterrupted sequence of between 5 and 7 a.a of any of the sequences of SEQ.IDs 1-4 which includes a positively charged a.a at its end. The positively charged a.a is preferably Lysine (indicated as K in the sequences) but may also be Arginine (R) or Histidine (H).

The continuous sequence can be part of a longer peptide of up to 10 a.a., wherein the continuous sequence is situated anywhere in the peptide. In case the peptide consists of more than 7 a.a, wherein the continuous sequence is at one of the peptides ends, said positively charged a.a will be at the open end of the sequence (which is not connected to the additional a.a of the longer peptide) so that the large peptide comprises a positively charged a.a at one of its ends . Wherein the continuous sequence is in the middle of the peptide, the peptide comprises at least one additional positively charged a.a. at one of its ends in addition to the positively charged a.a of the continuous sequence.

Analogues of the above peptides are also within the scope of the present invention. Such analogues are peptides which comprise no more than 10 a.a including at least 5 a.a which have the same sequence as one of the above mentioned continuous sequences but in which one or two a.a are conservatively replaced, as this term is defined below. The analogues also comprise at least one positively charged a.a at their end and essentially maintain the activity of the peptides as this term is defined below.

The term *"essentially maintains the binding characteristics"* refers to a peptide which level of binding to the tested sample is at least 50%, preferably 70%, most preferably 90% or more than 100% of the level of binding of the peptide to the same tested sample as determined by the same binding assay.

By a preferred embodiment, the invention provides a peptide which binds to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than it binds to a body fluid sample obtained from a non-schizophrenic individual said peptide selected from the group consisting of:

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4)

or analogs of said peptides in which no more than two a.a are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptides.

By a most preferred embodiment, a peptide is provided which binds to body fluid samples obtained from a schizophrenic patient substantively higher than it binds to a body fluid sample obtained from a non-schizophrenic individual having the amino acid sequence LVVGLCK (SEQ ID NO. 1).

By an additional aspect of the invention, a peptide is provided which binds to a body sample obtained from a schizophrenic patient substantively higher than it binds to a sample obtained from a non-schizophrenic individual, wherein the peptide binds antibodies that are capable of specific binding to a peptide having the amino acid LVVGLCK. Several non limiting examples of such peptides are the following:

i. KLVVGLC (SEQ ID NO. 2)
ii. LVVGLMK (SEQ ID NO. 3)
iii. KLVVGLM (SEQ ID NO. 4)

or analogs thereof in which no more than two a.a are conservatively replaced and which maintain the binding characteristics of the peptides.

The letters used above and throughout the present description to denote specific amino acids (a.a.) are in accordance with the one letter a.a. symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

In view of the fact that the peptides of the invention are the putative natural binding sites for autoantibodies found in elevated levels in body fluids of schizophrenic patients, and due to their high purity and high activity, these peptides are most useful for the diagnosis of schizophrenia. Thus, by an additional aspect,

the present invention provides an assay for the diagnosis of schizophrenia in an individual comprising the following steps:

(a) obtaining a body fluid sample from said individual being a blood sample, platelet-associated antibodies (PAA) containing fraction thereof, or a fraction containing PAA shed from the platelets;

(b) contacting said sample with a peptide being no more than 10 amino acids (a.a.) long and comprising a continuous sequence of at least 5 amino acids included in any one of the following sequences :

i. LVVGLCK (SEQ ID NO. 1)

ii. KLVVGLC (SEQ ID NO. 2)

iii. LVVGLMK (SEQ ID NO. 3)

iv. KLVVGLM (SEQ ID NO. 4);

said continuous sequence consisting of at least one positively charged a.a. at one end of said sequence; and said peptide comprising at least one positively charged a.a at its end being the positively charged a.a of said continuous sequence or at least one additional positively charged a.a.;

or analogs of said peptide being no more than 10 a.a long and in which no more than two a.a of said continuous sequence are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptide.

(c) determining the level of binding of said peptide to said sample, a level of binding substantively higher than the level of binding of said peptide to a sample obtained from a non-schizophrenic individual indicating that said tested individual has a high likelihood of having schizophrenia.

By a preferred embodiment, the peptide of step (b) is selected from the group consisting of:

i. LVVGLCK (SEQ ID NO. 1)

ii. KLVVGLC (SEQ ID NO. 2)

iii. LVVGLMK (SEQ ID NO. 3)

iv. KLVVGLM (SEQ ID NO. 4)

or analogs thereof in which no more than two a.a are conservatively replaced and which essentially maintain said peptide's binding characteristics.

By a preferred embodiment, the peptide of step (b) has the amino acid sequence LVVGLCK.

By an additional aspect, the peptides in step (b) are such which bind antibodies which bind peptides having the amino acid sequence LVVGLCK or analogues thereof.

Use of the peptides of the invention and analogues thereof as defined above and below for the preparation of a diagnostic composition for diagnosis of schizophrenia in an individual is also within the scope of the present invention.

By an additional aspect, the invention provides a kit useful in the above assay, said kit comprising a support comprising one or more peptides of the invention immobilized onto it, an anti-human immunoglobulin (hIg) antibody or fragment thereof, or one or more non-based peptides conjugated to a detectable marker which bind to antibodies present in the tested sample, reagents required for carrying out the detection assay wherein said peptides bind to antibodies present in a tested sample as well as instructions for use.

Wherein the detection of the binding of the peptides of the invention to the tested sample is by an anti-hIg antibody the anti-hIg antibody may be conjugated to a detectable marker or alternatively, the kit may also comprise a second type of antibodies directed against said first antibodies, wherein the second antibodies are conjugated to a detectable marker.

By one embodiment, the binding of the peptide of the invention to the tested sample is detected using second non-bound peptides complexed with a detectable marker, said second peptides capable of binding to the antibodies present in the tested sample. In accordance with this embodiment, the detection is achieved by a double antigen sandwich text which may be performed as a one step assay or as a two step assay. Wherein the detection is performed by the double antigen sandwich text assay, the kit of the invention will include such

peptides conjugated to a detectable marker instead of the anti-human immunoglobulin antibody.

The assay of the invention may be used as a single test for detecting a high likelihood of schizophrenia in an individual. However, in accordance with an additional aspect of the Invention, the peptides and assay may be used as a confirmatory diagnostic tool. Thus, for example, wherein a high likelihood of schizophrenia is determined in an individual by methods used to date (mainly psychiatric evaluation, as mentioned above), this could be reaffirmed (or, alternatively, re-evaluated) by using the assay of the Invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphical representation showing a surface presentation of enolase which demonstrates that the prior art peptide cannot be the natural binding site for autoantibodies. As can be seen, only arginine R402 (arrow) would be available to binding antibodies wherein the remaining part of the peptide is buried within the protein.

Fig. 2 is a graphical representation of the putative epitope of the peptides of the invention, which consists of six neutral amino acids (L413, L183, L409, L406, P400 and A401) which are surrounded by four positively charged amino acids (R414, R402, R184 and K194).

Fig. 3 is a schematic representation showing the level of binding of plasma samples from 39 schizophrenic patients (filled squares) and 50 non schizophrenic individuals (open squares) with the biotinylated peptide LVVGLCK in an enzyme immunoassay.

Fig. 4 is a schematic representation showing an inverse correlation between the O.D. values measured in the enzyme immunoassay described above in samples obtained from schizophrenic patients and the duration of the disease in these patients.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides short highly purified and highly active peptides, which comprise a putative natural epitope of the peptides to which autoantibodies found in elevated levels in schizophrenic patients, bind. The short length of these peptides (7-10 amino acids) and their structure, which is a structure, which can be exposed on the surface of the enolase enzyme in a manner, which enables the autoantibodies to bind to it, render these peptides to be most useful in the diagnosis of schizophrenia. Thus, although the peptides of the prior art (WO 99/51725) were able to bind to autoantibodies present at higher levels in schizophrenic patients as compared to non-schizophrenic individuals, the peptides of the present invention, having the above characteristics enable to detect an individual having a high probability of having schizophrenia more effectively.

The binding activity of the peptide of the invention to various antibodies may be determined by any of the methods known *per se* such as ELISA or Western Blotting. For example, a tested peptide may be analyzed for its binding activity to antibodies by subjecting it to polyacrylamide gel electrophoresis, blotting it onto PVDS membranes which are then reacted with a body fluid sample of the tested sample and compared to their reaction with a sample obtained from a non-schizophrenic individual.

The extent of binding of the peptides of the invention to PAA can be determined by using any detection system known in the art such as antibodies against human immunoglobulin or fragments thereof linked to a detectable marker. The marker may be a radioactive group, a fluorescent group, an enzyme capable of catalyzing a reaction yielding a detectable product, a biotin group capable of being detected by avidin, etc.

By a preferred embodiment, the extent of binding of the peptides of the invention to the tested sample is carried out using an enzyme immunoassay in which the peptides are labeled with biotin and bound to streptavidine coated tubes as explained below.

In accordance with the invention, it has been found that the position of the positive charge in the peptides may be either at the beginning or at the end of the peptide. This indicates that the peptides exist in an open ring form. In addition it was found that cysteine does not have importance in the sequence of the peptides since substitution of cysteine with Methionin retained the activity of the peptides. Thus, as explained above, the continuous sequence of a.a comprised within the peptide of the invention consists of a positively charged a.a at its end. If the continuous sequence is at one of the ends of the whole peptide, this positively charged a.a will be at one end of the whole peptide. Alternatively, if the continuous sequence is not at the end of the whole peptide, the whole peptide will comprise an additional positive charge at one of its ends.

It is known that the antibody binding site is usually consisted of at least five amino acids. Thus, the peptides of the Invention and their analogues comprise at least five amino acids. The five amino acids will be such which appear consecutively in one of the continuous sequences of the invention.

In order to maintain the binding characteristics of the peptide, the analogues of the invention comprise no more than two a.a. substitutions which are conservative substitutions. These are substitutions in which an amino acid of one class is replaced by an amino acid of the same class, where a class is defined by common physiochemical amino acid chain properties such as charge, size or hydrophobicity. Amino acids of the same class are characterized by high substitution frequencies in homologous proteins found in nature (as determined, for example, by a standard Dayhoff frequency exchange matrix).

Thus for example the leucine positioned in the first position of the amino acid sequence of a peptide of the invention may be conservatively substituted by the amino acids glycine or valine, which belong to the same family of amino acids, without altering the binding activity of the peptide. The positively charged a.a at the end of the peptide of the invention may be conservatively replaced by another positively charged a.a. A person versed in the art will have no difficulty in determining by which amino acid each of the amino acids of the peptide may

be replaced in accordance with the known grouping of amino acids into families as may be found, for example, in Molecular Biology of the Cell Editors Alberts B. *et al.*, Garland Publishing, Inc., New York and London, 2nd Edition, 1989, pages 54-55.

The peptides of the invention may also be chemically modified. In such a chemically modified peptide at least one of the amino acid residues may be modified either by a natural process such as post-translational modification or by chemical modification techniques which are well known in the art. Examples of chemical modifications are acetylations and glycolysations, glycosamine-glycinations, ADP-ribosylations, covalent attachment of a lipid or a lipid derivative, methylation, myristylation, pegylation, phosphorylation, etc. The chemical modification may be at the peptide's amino end or at the carboxy end.

The peptides of the invention may additionally have a non peptide component attached such as for example a macromolecular carrier group which may covalently be attached to the amino or carboxy side of the peptide. Such a carrier may for example be polyethylene glycol, carbohydrates or lipid fatty acid conjugates.

All the above describe changes in the peptides of the invention that may result in increased stability, bioavailability or activity of the peptides of the invention.

The peptide of the invention may be obtained by enzymatic digestion (e.g. using Clostrapain) or chemical (CNBr) digestion of a longer protein. In such a case, the resulting peptides are separated by methods known in the art such as by RP-HPLC and the separate peptides may then be used for sequencing (e.g. by Eurosequence b.v. (Nijenborgh 4; 9749 Gronigen; The Netherlands)) and analyzed for their binding capability to antibodies as described above.

The short length of the peptides of the Invention (5 to 10 a.a) renders them as very good candidates for synthesize by methods known in the art such as on Abimed 522 at a 10 μmol scale by Eurosequence b.v. (see detailed explanation in

the examples below). The binding activity of the newly synthesized peptides will be determined using any of the assays mentioned above.

Another advantages of the present invention, which is based on the fact that the peptides of the invention are short, purified and active peptides, is that the *"body fluid"* sample of the individual to be tested may be a blood plasma or serum sample which is relatively easy to obtain and prepare. However, at times it may be advantageous to perform the assay of the invention on a blood sample obtained from the tested individual which is a PAA containing fraction obtained from the sample by any of the methods known in the art, such as by obtaining a platelet-rich plasma and isolating PAA therefrom. In accordance with the invention, the sample may also be any other body fluid sample obtained from the tested individual including a whole blood sample, or any other body fluid sample containing PAA, e.g. saliva, cerebrospinal fluid, etc. Chemical analysis as well as immunoactivity assays in accordance with the invention showed that the peptide having the amino acid sequence LVVGLCK had the highest purity of synthesis and could spontaneously form dimers thus doubling the epitope per binding site of streptavidine and enhancing the binding potential of PAAs to this peptide. Therefore, in accordance with the invention, a peptide having the above sequence or a peptide comprising this sequence are preferred for use in the diagnosis of schizophrenia in accordance with the invention.

EXAMPLES

The invention will now be demonstrated by the following non limiting Examples with reference to the figures.

Materials and Methods

1. Patients and control persons – Thirty-nine schizophrenic patients participated in this study. The following parameters were recorded: gender, age, duration of schizophrenia, number of hospitalizations, years of

education and the psychiatric status (PANSS). Fifty plasma samples were obtained from a local blood bank.

2. Plasma – Venous blood was drawn with heparin as coagulant from patients and control subjects. Plasma was obtained after centrifugation (4000 g; 15 mins. 4°C).

3. Peptide synthesis and biotin labeling – The peptides were synthesized on an Abimed 522 at a 10 micromol scale by Eurosequence b.v., (Nijenborgh 4: 9747 AG Groningen; The Netherlands) and labeled at the amino terminal with biotin containing a six carbon spacer. Peptide purity was routinely assessed by RP-HPLC and, when considered necessary, by laser desorption mass spectroscopy. The peptides were routinely dissolved in 1 ml water/DMF/DMSO (1:1:1; v/v/v).

4. Line blot – peptides were line blotted onto PVDF or nitrocellulose membranes. Membrane strips were incubated overnight with 0.5 ml plasma sample in 0.5 ml buffer (200 mM Tris; 0.3% Casein; 200 mM KCl; 10,6 mM phenol; 2.1 mM $CaCl_2$; 0.01% Triton X-100; pH 8.5) at room temperature under gentle shaking in BioRad incubation arrays. After three washings with PBS, strips were probed with Anti-human Fc (goat) conjugated to horseradish peroxidase (SIGMA; dilution 1:100) for 2 hours at room temperature under gentle shaking. Fast-DAB™ or 4-Chloro- naphthol (SIGMA) were used as color reagent to detect bound antibodies.

5. Enzyme immuno assay – Streptavidine coated tubes (Boehringer Mannheim; 80 nMol Streptavidine per tube) were coated with a tenfold excess of biotin-labeled peptide in 1 ml PBS for 3 days at 4°C, washed twice with 2 ml 1M NaCl, once with 2 ml water, dried and stored under vacuum at minus 18°C until use. For the test, 0.05 ml sample and 1 ml Incubation

Buffer (60 mM citric acid; 90 mM Na_2HPO_4; 168 mM NaOH; 200 mM NaCl; pH 7.7) are added to the peptide labeled tube and incubated for 1 hr at 37°C without shaking. The tubes were covered in order to prevent evaporation. The tubes were then washed three times with 2 ml 1 M NaCl solution for 15 min. under gentle shaking, 1 ml Anti-human Fc (goat) conjugated to horseradish peroxidase (SIGMA) added at a dilution of 1:2000 in POD Buffer (200 mM Tris; 0.3% Casein; 200 mM KCl; 10,6 mM phenol; 2.1 mM $CaCl_2$; 0.01% Triton X-100; pH 8.0) and incubated for 0.5 hr at 37°C without shaking. After 4 washings with 2 ml 1M NaCl solution, the color reaction was initiated by adding 1 ml Tetramethylbenzidine (TMB) Liquid Substrate System™ (SIGMA). The enzymatic reaction was stopped by adding 1 ml 0.5M H_2SO_4. The absorbence was read at 450 nm spectrophotometrically.

Example 1:

Four peptides in accordance with the invention, having one of the a.a. sequences:

(a) LVVGLCK
(b) KLVVGLC
(c) LVVGLMK
(d) KLVVGLM

were biotin-labeled and coated onto streptavidine coated tubes as described above. A pool of plasma samples was prepared from 5 schizophrenic patients and an additional pool of plasma was prepared from 5 non-schizophrenic individuals.

Results:

All of the four peptides of the invention bound to the plasma pool originating from schizophrenic patients to a higher extent than their binding to the control plasma sample obtained from non-schizophrenic individuals (as measured in the enzyme immunoassay described above which showed 0.5 O.D. in the

samples of the peptides with the control plasma samples and 1.6 O.D. in the samples of the peptides with the schizophrenia derived plasma pool).

Example 2: Diagnosis of schizophrenia using the peptide LVVGLCK

Of the four peptides of the invention, the highest purity of synthesis was found in the peptide LVVGLCK. In addition, these peptides were found to spontaneously form dimers, thus doubling the epitope for binding site of streptavidine.

Therefore, an experiment was carried out with the biotin-LVVGLCK as follows: the peptide was biotin-labeled and coated onto streptavidine coated tubes as explained above. Plasma samples of 39 schizophrenic patients and 50 control non schizophrenic individuals were tested with the coated peptide and the level of binding was determined by the enzyme immunoassay described above.

As seen in Fig. 3, the biotinylated peptide LVVGLCK bound to a higher extent to the plasma samples obtained from schizophrenic patients (filled squares) at a mean value and standard deviation of 1.47 ± 0.65 as compared to its binding to plasma samples from non schizophrenic individuals (open squares) with mean value and standard deviation amounting to 0.46 ± 0.21. The difference of binding of this peptide to the two groups was statistically highly significant (1.1×10^{-11} using Student's T-test).

Example 3: Analysis of schizophrenia on the basis of the peptide of the invention as compared to acceptable psychiatric analysis

By further analysis of the results described in Example 2 above, with other recorded psychiatric parameters of the patients, an inverse correlation was found between the duration of schizophrenia and the level of binding to the peptide of the invention measured as optical density (O.D.). A geometric fit ($y = ax^{bx}$) was applied with $a = 1.9376$ and $b = 0.000798$ as coefficients (Fig. 4). Summarizing Figs. 3 and 4, the following results shown in Table 1 below can be reached by comparing the biochemical test with the psychiatric evaluation.

Table 1

Psychiatric Evaluation		Biochemical Test			
		1-15 years disease	16-30 years disease	1-30 years disease	Controls
No. of tested individuals	N = 39	N = 23	N = 16	N = 39	N = 50
Schizophrenic:	39	19	7	26	0
Borderline:	0	1	6	7	6
Negative	0	3	3	6	44

This takes the following definitions into account:

Negative: 0 until mean + 1s

Borderline: Between mean + 1s until mean + 2s

Positive: Higher than mean + 2s

In conclusion, a sensitivity of better than 80% and a specificity of better than 90% seem to be feasible. However, it should be noted that these definitions are arbitrary and may, therefore, be changed depending whether the emphasis is more on specificity or on sensitivity.

CLAIMS:

1. A peptide which binds to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than its binding to a body fluid sample obtained from a non-schizophrenic individual, said peptide being no more than 10 amino acids (a.a.) long and comprising a continuous sequence of at least 5 amino acids included in any one of the following sequences :

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4);

said continuous sequence consisting of at least one positively charged a.a. at one end of said sequence; and said peptide comprising at least one positively charged a.a at its end being the positively charged a.a of said continuous sequence or at least one additional positively charged a.a.;

or analogs of said peptides being no more than 10 a.a long and in which no more than two a.a of said continuous sequence are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptides.

2. A peptide which binds to a body fluid sample obtained from a schizophrenic patient at a substantively higher level than its binds to a body fluid sample obtained from a non-schizophrenic individual said peptide selected from the group consisting of:

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4)

or analogs of said peptides in which no more than two a.a are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptides.

3. A peptide which binds to body fluid samples obtained from a schizophrenic patient substantively higher than it binds to a body fluid sample obtained from a

non-schizophrenic individual having the amino acid sequence LVVGLCK (SEQ ID NO. 1).

4. A peptide which binds to a body sample obtained from a schizophrenic patient substantively higher than it binds to a sample obtained from a non-schizophrenic individual, wherein the peptide binds antibodies that are capable of specific binding to a peptide having the amino acid LVVGLCK (SEQ ID NO.1).

5. A peptide according to Claim 4, having the amino acid sequence KLVVGLC (SEQ ID NO. 2).

6. A peptide according to Claim 4, having the amino acid sequence LVVGLMK (SEQ ID NO. 3).

7. A peptide according to Claim 4, having the amino acid sequence KLVVGLM (SEQ ID NO. 4).

8. An assay for the diagnosis of schizophrenia in an individual comprising the following steps:

(a) obtaining a body fluid sample from said individual being a blood sample, PAA-containing fraction thereof, or a fraction containing platelet-associated antibodies (PAA) shed from the platelets;

(b) contacting said sample with a peptide being no more than 10 amino acids (a.a.) long and comprising a continuous sequence of at least 5 amino acids included in any one of the following sequences :

i. LVVGLCK (SEQ ID NO. 1)

ii. KLVVGLC (SEQ ID NO. 2)

iii. LVVGLMK (SEQ ID NO. 3)

iv. KLVVGLM (SEQ ID NO. 4);

said continuous sequence consisting of at least one positively charged a.a. at one end of said sequence; and said peptide comprising at least one positively charged a.a at its end being the positively charged a.a of said continuous sequence or at least one additional positively charged a.a.;

or analogs of said peptides being no more than 10 a.a long and in which no more than two a.a of said continuous sequence are conservatively substituted, said analogues essentially maintaining the binding characteristics of the peptides.

(c) determining the level of binding of said peptide to said sample, a level substantively higher than the binding level of said peptide to a sample obtained from a non- schizophrenic individual indicating that said tested individual has a high likelihood of having schizophrenia.

9. A method according to Claim 8, wherein the peptide of step (b) is selected from the group consisting of:

i. LVVGLCK (SEQ ID NO. 1)
ii. KLVVGLC (SEQ ID NO. 2)
iii. LVVGLMK (SEQ ID NO. 3)
iv. KLVVGLM (SEQ ID NO. 4)

or analogs thereof in which no more than two a.a are conservatively substituted and which essentially maintain said peptides' binding characteristics.

10. A method according to Claim 8, wherein the peptide of step (b) has the amino acid sequence LVVGLCK (SEQ ID NO.1).

11. A method according to Claim 8, wherein the peptide in step (b) is such which binds antibodies which bind peptides having the amino acid sequence LVVGLCK (SEQ ID NO. 1) or analogs thereof in which no more than two a.a. are conservatively substituted.

12. A kit for use in the diagnosis of schizophrenia comprising a support comprising one or more peptides of Claim 1, immobilized onto it, anti-human immunoglobulin (hIg) antibody or fragment thereof, reagents carrying a detection assay whereby said peptides bind to antibodies present in a tested sample, as well as instructions for use.

13. A kit according to Claim 12, wherein said anti-hIg antibody is complexed to a detectable marker.

14. A kit according to Claim 12, wherein instead of said anti-hIg antibody, the kit comprises one or more non-bound peptides which bind to antibodies present in a tested sample, said peptides complexed to a detectable marker.

15. Use of any of the peptides of Claim 1 for the preparation of a diagnostic composition for the diagnosis of schizophrenia in an individual.

16. The assay of Claim 8, for use in confirming a high probability of Schizophrenia in an individual determined by at least one other diagnostic assay.

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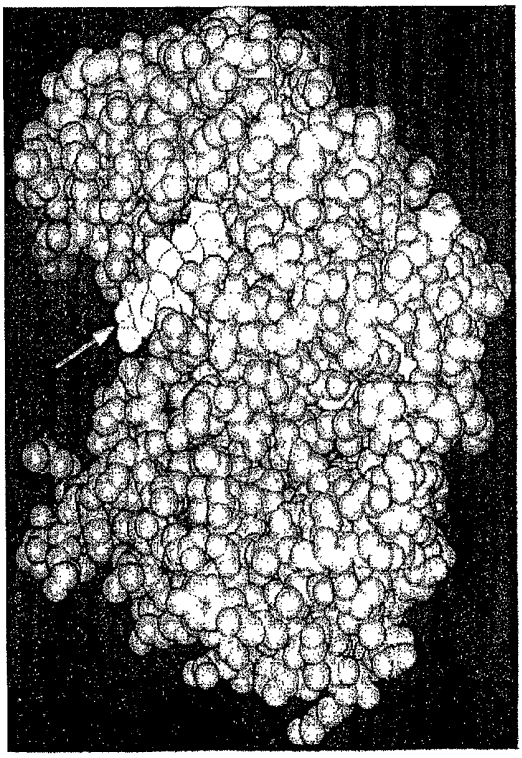

FIG.1

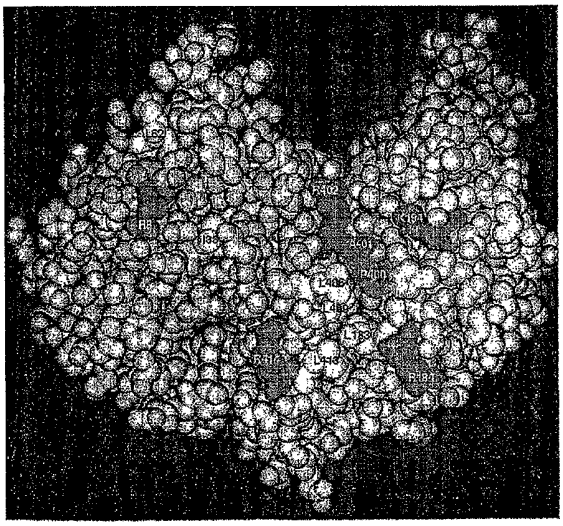

FIG.2

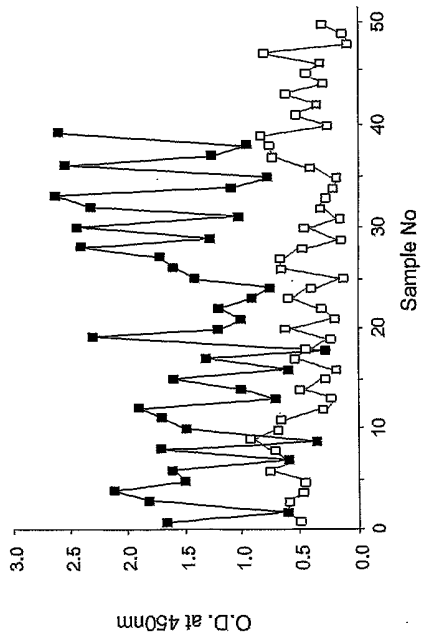

FIG. 3

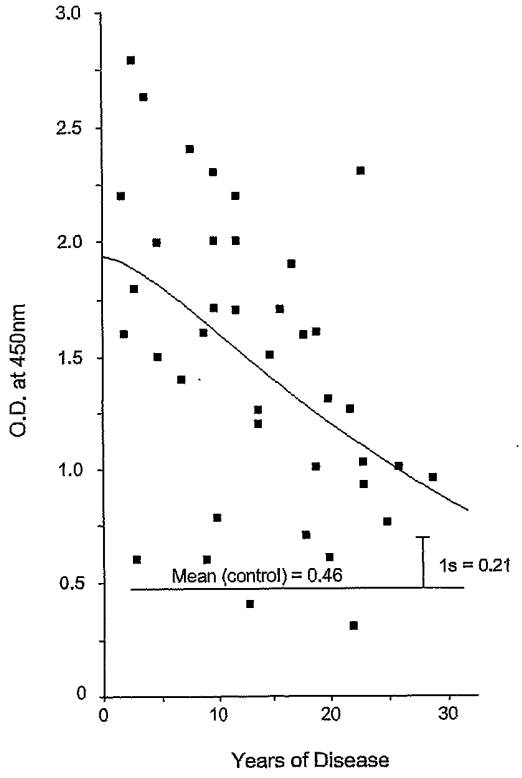
3.0
2.5
2.0
1.5
1.0
0.5
0
O.D. at 450nm
0
10
20
30
Years of Disease
Mean (control) = 0.46
1s = 0.21

FIG. 4

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 02/00233

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 C07K7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE, PAJ, CHEM ABS Data, BIOSIS, WPI Data, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 51725 A (DECKMANN MICHAEL ;SHINITZKY MEIR (IL); YEDA RES & DEV (IL)) 14 October 1999 (1999-10-14) cited in the application the whole document	1-16
X	WO 00 06723 A (FITZER ATTAS CHERYL ;FRIDKIN MATITYAHU (IL); BAR HAIM EREZ (IL); C) 10 February 2000 (2000-02-10) page 30; table 3	1,2,8,9, 11-16
Y	WO 95 23970 A (YEDA RES & DEV ;RYCUS AVIGAIL (IL); SHINITZKY MEIR (IL); DECKMANN) 8 September 1995 (1995-09-08) the whole document	1-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 January 2003	28/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Pinheiro Vieira, E

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INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/IL 02/00233

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	VERMUYTEN K: "DETECTION OF NEURON SPECIFIC ENOLASE CONCENTRATIONS IN CEREBROSPINAL FLUID FROM PATIENTS WITH NEUROLOGICAL DISORDERS BY MEANS OF A SENSITIVE ENZYME IMMUNOASSAY" CLINICA CHIMICA ACTA, AMSTERDAM, NL, vol. 187, no. 2, 1990, pages 69-78, XP009003003 ISSN: 0009-8981 the whole document	1-16
Y	WO 92 18644 A (UNIV COLUMBIA) 29 October 1992 (1992-10-29) the whole document	1-16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IL 02/00233

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(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZM,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,OM,PH,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TN,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZM,ZW

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Ｆターム(参考) 4H045 AA10 AA30 BA14 CA42 DA86 EA50 FA20

专利名称(译)	用于诊断精神分裂症的新型肽		
公开(公告)号	JP2004533420A	公开(公告)日	2004-11-04
申请号	JP2002573801	申请日	2002-03-21
[标]申请(专利权)人(译)	耶达研究及发展有限公司		
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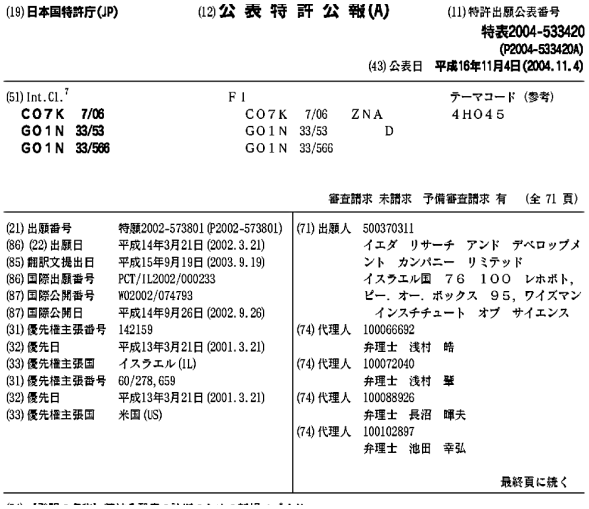
发明人	デックマン、ミヒャエル		
IPC分类号	G01N33/53 C07K7/06 C07K14/47 G01N33/566		
CPC分类号	C07K14/4713		
FI分类号	C07K7/06.ZNA G01N33/53.D G01N33/566		
F-TERM分类号	4H045/AA10 4H045/AA30 4H045/BA14 4H045/CA42 4H045/DA86 4H045/EA50 4H045/FA20		
代理人(译)	池田幸		
优先权	142159 2001-03-21 IL 60/278659 2001-03-21 US		
其他公开文献	JP5048205B2		
外部链接	Espacenet		

摘要(译)

提供与从精神分裂症患者获得的体液样品结合的短肽，其水平显着高于从非精神分裂症患者获得的体液样品的结合水平。该肽的长度为10个氨基酸或更短，并且包含至少5个氨基酸的连续序列，其在其一个末端包含至少一个带正电荷的氨基酸。因此，所提供的肽是精神分裂症患者中高水平发现的自身抗体的推定结合位点，可用于精神分裂症的诊断。



(19)日本国特許庁(JP)　(12)公表特許公報(A)　(11)特許出願公表番号
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(P2004-533420A)
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G01N 33/53	G01N	33/53 D	
G01N 33/566	G01N	33/566	

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(33)優先権主張国	米国 (US)

(71)出願人　500370311
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