Thank you!

The Characteristics of Epdemiology on W/Beijing lineage *Mycobacterium tuberculosis* isolates in Changping, Beijing

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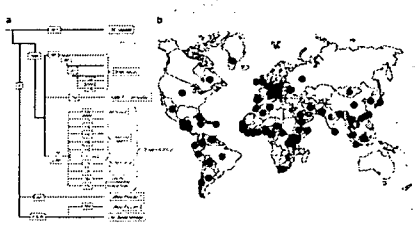
Background

- Recently it was identified some large sequence polymorphisms (LSPs) through microarray approaches. Most of LSPs are of unique event polymorphism (UEP). Thus these LSPs not only were used to class *Mycobacterium tuberculosis*, but also to investigate the M.tb phylogeny.
- Peter Small et al analysis the global M.tb (875 strains) by twenty LSPs and demonstrated that global population structure of M.tb is defined by six phyogeographical lineages, each associated with specific human populations.

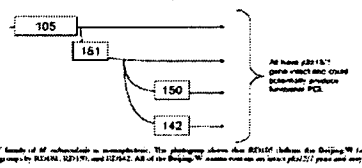
Fig. 1. The global population structure and geographical distribution of M. tuberculosis



Gagneux, Sebastien et al. (2006) Proc. Natl. Acad. Sci. USA 103, 2869-2873

PNAS

- In 2003, Kristin Kremer had renamed Beijing Genotype M.tb into *Mycobacterium tuberculosis* Beijing/W lineage. Beijing/W lineage belonged to East Asia lineage. Our research found , besides the other lineages, the Beijing/W lineage was dominant (64.9%) in china. Consequently the Beijing /W lineage can be futher subdivided into at least two subgroup by LSPs (LSPs also called RD region of difference)



- Chang ping was one of districts of Beijing, and lies its north.There are 1,320,000 population and 130 TB cases every year. In addition, like other districts, the TB incidence has potential increasing danger because of immigrant and TB drug resistance.

Object

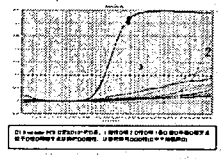
- Descript the phylogeny tree of *Mycobacterium tuberculosis* on Changpin, Beijing.
- Analysis the associations of sub-groups of Beijing/W lineage with patient's drug resistance, place of birth, BCG vaccination and age.
- In a word, we hope to explored the Characteristics of Epdemiology on W/Beijing lineage *Mycobacterium tuberculosis* strains isolated from Changping, Beijing.

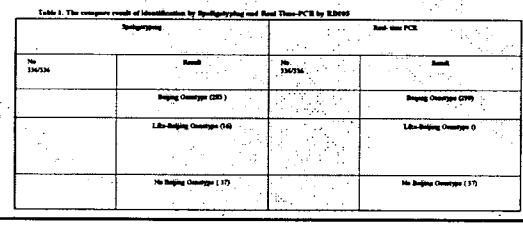
Materials and Methods

- Three hundreds thirty-six *M.tb* isolated strains were collected in succession from first Jan 2004–31th December 2006 in Changping, Beijing.
- Beijing lineage *M.tb* was defined by Spoligotyping and RD105 using Real Time-PCR .
- Beijing lineage *M.tb* was further subdivided into two sub-groups atypical Beijing strains and W strain/typical family strains by RD181 using Real-time PCR and by multiplex PCR method.
- W strain/typical family strains were finally divided by RD150 and RD142.
- The associations of sub-groups (atypical Beijing strains and W strain/typical family strains) with patient's drug resistance, place of birth, BCG vaccination and age were assessed by the χ^2 test.

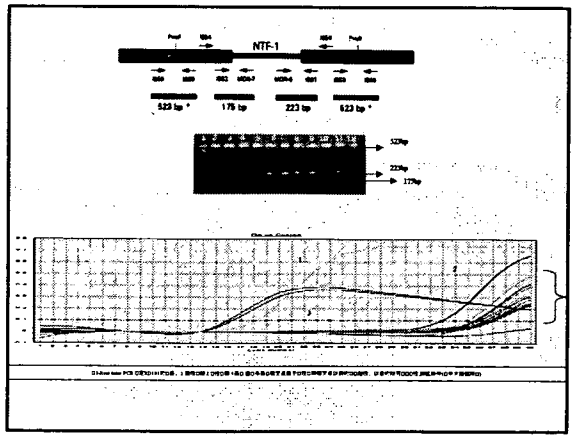
Result

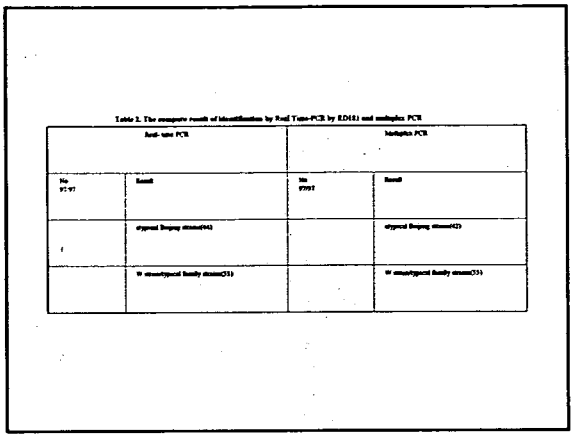
- Of 89.0% (299/336) strains were W/Beijing lineage, and 11.0%(37/336) were not W/Beijing lineage by the two methods.
- The identification result using Spoligotyping and Real-Time PCR by RD105 was consistent.





- In W / Beijing lineage Mycobacterium tuberculosis , 15.7%(47/299) were "old' atypical Beijing strains, presence RD181.
- 84.3%(252/299) were "modern" W strain/typical family strains, deleting RD181.
- The identification result using Real-Time PCR by RD181 and multiplex PCR was consistent.





- In W strain/typical family strains, 168 and 173 *M.tb* strains was deleted in RD150 and RD142 respectively.

Conclusion

- Descript the phylogeny tree of *M.tb* on Changpin, Beijing, where the W/Beijing lineage strains were dominated.
- However the W strain/typical Beijing family strains were of preponderance again.

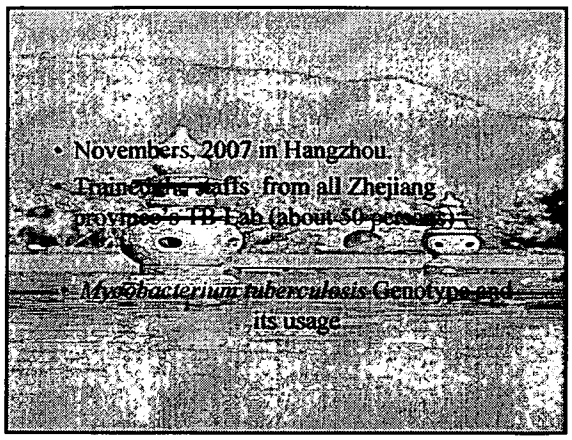
Conclusion

- No significatly associations of the two sub-groups of Beijing/W lineage with patient's drug resistance, place of birth, BCG vaccination and age.

Hypothesis

- In Beijing area, W/Beijing lineage *Mycobacterium tuberculosis*, specially W strain/typical Beijing family strains were dominated, and infected hosts, regardless their place of birth, BCG vaccination and age.

Developing *Mycobacterium tuberculosis* Genotype in China



- December, 2007 in Beijing.
- Trained the staffs from all of our country's TB Lab (about 100 persons)
- *Mycobacterium tuberculosis* Genotype and its usage

Acknowledge

- Li Chuanyou
- Li Yi
- Gao Feng
- Sun Zhaogang
- Qiu Yunqing
- Tian Miao
- Zhang Xuxia
- Gao Tiejie
- Zhang Zhiguo
- Zhou Hui
- Zhao Yanlin

Establishment of standard VNTR TB typing method for Beijing genotype

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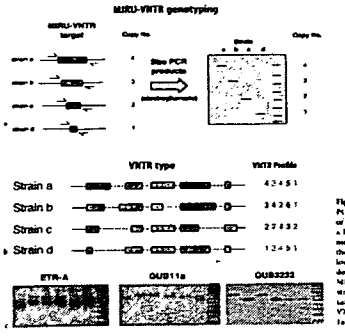
Molecular Epidemiology Division,
Mycobacterium Reference Center,
The Research Institute of Tuberculosis
Japan Anti-Tuberculosis Association

Variable Numbers of Tandem Repeats



Comparing the cluster rates

Method	Cluster
IS*6110* RFLP	41.2% (40/97)
VNTR	
12 MIRU	55.7% (54/97)
12 MIRU + 4 ETR	50.5% (49/97)

325 isolates were collected from whole Japan

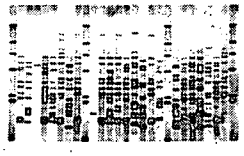
(1) IS*6110* RFLP analysis

(2) Spoligotyping

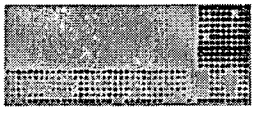
(3) VNTR analysis

16 loci : MIRU-VNTR (12 loci)とETR (4 loci)
19 loci : QUB (5 loci) and the other loci

IS6110 RFLP



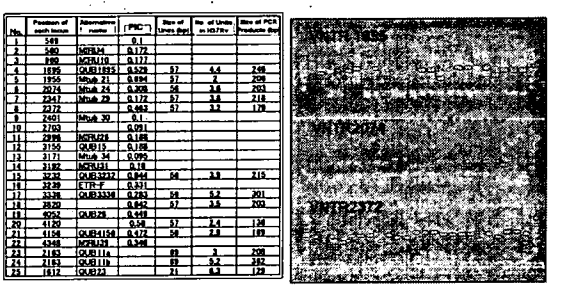
Spoligotyping



Cluster rates of RFLP and spoligotyping

Typing method	Total no. of type patterns	No. of unique types	No. of clusters	No. of clustered isolates (%)	Maximum no. of isolates in a cluster	HGDI*
IS*6110* RFLP	283	265	18	60 (18.5)	8	0.998
spoligotyping	45	32	13	293 (90.0)	228	0.501

Promising VNTR loci for genotyping of Beijing strains



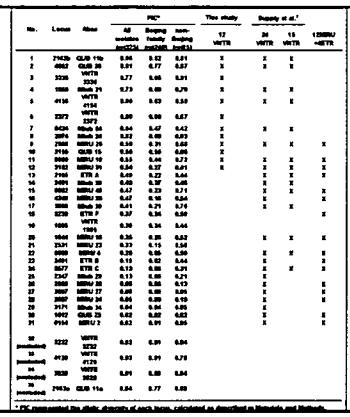
Beijing type (n=21)

Smittipat et al, JCM 43, 5034-43 (2005)

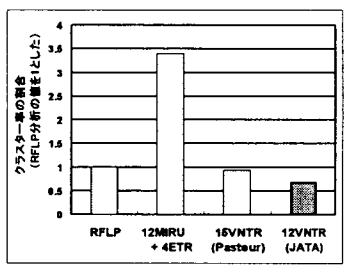
Distribution of copy numbers of each locus in VNTR analyses

VNTR locus	No amplification	Plural PCR product	Copy number of repetitive unit[s] 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over	PIC
2163a																			
(QUB-11a)	5				10	27	5	12	27	10	30	103	51	15	5		7	19	0.843
2164a																			
(QUB-11b)	3	1		6	29	56	35	35	35	75	30	6	1	2	1			1	0.857
3156																			
(QUB-15)				8	23	62	186	34	3										0.586
1612																			
(QUB-23)						1		2	321	1									0.024
4052																			
(QUB-26)	1			2	8	36	6	15	23	27	58	114	19	11	5	1			0.810
1886					5	48	14	255	3	4		4						2	0.363
1955				4	29	41	141	71	31	2		1	1	3	1				0.731
2074				19	83	179	39	6											0.623
2372				1	22	79	156	58	7	3									0.679
2401					6	114	4	209	1										0.482
3232		1	1	1	8		2	23	25	5	6	6	17	18	27	36	22	40	96	0.932
3326		1	1	5	19	9	20	188	69	28	16	7	17	9	7	2	2	1	2	0.772
3628		1			2	9	19	32	9	6	9	12	14	8	31	51	44	37	50	0.915
4138		3	1	19	28	23	27	11	19	21	28	29	44	21	19	9	9	14	0.927
4156		1			12	96	75	131	19										0.694
0424	1				18	72	61	171	4	1									0.636
2347	1					13	8	382		1									0.126
3171					1	2	319		3										0.041
3680					6	17	348	19	21	7	7								0.406

Comparing of PIC in each locus



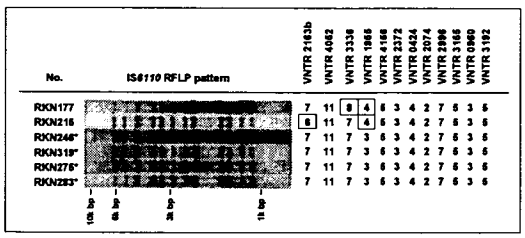
Comparing cluster rates in RFLP, Supply(15) and JATA(12) analyses



Comparing the maximum size of cluster in each methods

Size	RFLP	12MIRU+4ETR	15VNTR (Supply)	12VNTR (JATA)
2	10	29	16	14
3	3	9	3	3
4		3	1	1
5	1	1	1	
6	3	1	1	
7		1		
8	1			
9				
10				
17		1		
25				
34		1		
44		1		

JATA (12) –VNTR analysis of the isolates clustering in RFLP



Conclusion

(1)By analyzing the TB collected from whole Japan, 70 % of TB isolates were Beijing genotype.

(2) The percentage of cluster rate in IS*6110* RFLP was 18.5%.

(3) We established new promising loci of VNTR analyses for Beijing strains.

The discrimination power of JATA(12)-VNTR analysis is higher than that of IS*6110* RFLP and Supply (15).

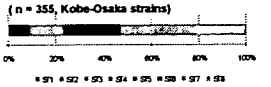
China-Korea-Japan Joint Research Conference
(JATA-RIT, Tokyo, February 13-14, 2008)

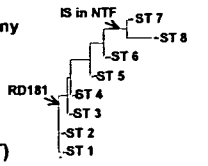
Population Structure Analysis of *M. tuberculosis* Beijing Family Implies Association of Certain Sublineages and Multidrug Resistance

Tomotada Iwamoto Ph.D.
Kobe Institute of Health

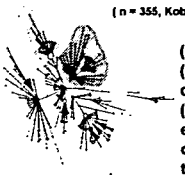
Population structure analysis of Beijing genotype TB

10 sets of synonymous SNPs
Considered as gold standard for phylogeny





15-VNTR (genotyping, tree by MST)
Diversity of Beijing TB in the population



Key findings:
(1)Ancient type dominates in Japan.
(2)Each sub-lineage consists diverse clones identified by 15-VNTR.
(3)BJ-TB in Japan seems to become endemic separately from recently observed global trend, i.e., modern type dominates the population.

Do individual sub-lineages have evolved unique pathogenic characteristics ????

- Genotypic approach
  Comparative Genomic Hybridization
  (By Dr. Wada and Dr. Maeda)
- Phenotypic approach
  Evaluating spontaneous mutation rate
  (By S. Yoshida)
- Molecular epidemiological approach
  Association of each sub-lineage with drug resistance.

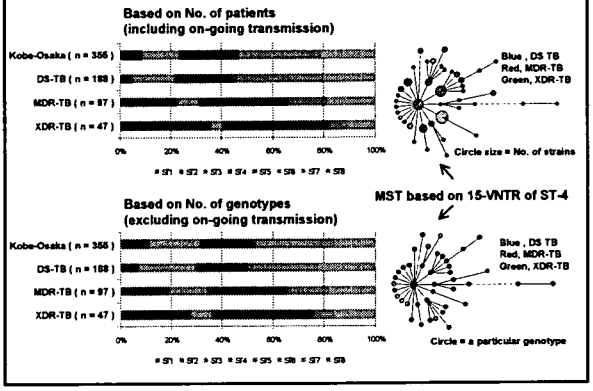
Study design

Strains.
188 drug susceptible (DS) strains (Jan 1, 03 – Aug 31, 03)
97 multidrug-resistant (MDR) strains(Jan 1, 01 – Dec. 31, 06)
(47 extensively drug-resistant (XDR) strains are included)
All strains obtained from individual patients at one hospital.

Genotyping.
Supply's 15-locus VNTR.

Phylogenetic analysis.
- 10 synonymous single nucleotide polymorphisms (SNPs).
- Large sequence polymorphisms (RD181, RD150, RD 142).
- IS*6110* insertion in NTF region.
- 4 SNPs in putative mismatch repair genes.

Frequency of occurrence of DS-, MDR-, XDR-TB



What is a driving force for overabundance of MDR / XDR TB in certain sublineages ????

- Any genetic event ?
  Mutation in the mismatch repair genes has been hypothesized to enhance the ability to acquire drug resistance (by Rad, et al. EID 838-845, 2003)
  →Putative mismatch repair genes (4 SNPs)

- On-going transmission ?
  →Clustering rate
  →*katG* S315T mutation (maintain transmissibility)
  The prevalence is high in countries with a high prevalence of MDR-TB

- Clonally evolution ?
  → Clinical & demographic data, diversity in genotype

Association of DNA repair genes with drug resistance

sSNPs	No. of strains			Putative mismatch repair genes				LSP	
	DS	MDR	XDR	*mut*T2	*mut*T4	*ogt* (12)	*ogt* (37)	RD181	RD150
ST 1	1	0	0	Wt	Wt	Wt	Wt	+	+
ST 8	9	10	4	Mt	Mt	Mt	Wt	-	+

The presence or absence of putative mutator genes (*mutT* and *ogt* genes) mutations are more likely to be genetic markers for the phylogeny with no effect on the ability to acquiring drug resistance.

- No discrepancy in the process of accumulation of mutations between DS- and MDR-TB.
- The mutator phenotype does not appear to increase prevalence of drug resistance.

Clustering rate and *katG* S315T mutation

ST	No. of clustered strains / total (%)			*KatG* S315T mutation	
	DS	MDR	XDR	MDR (%)	XDR (%)
2	2 / 9	14 / 22	12 / 17	15	14
	(22.2)	(63.6)	(70.6)	(68.2)	(82.4)
	(63.6)	(64.7)	(65)	(50.0)	(60.0)
8	4 / 9	9 / 10	3 / 4	1	0
	(44.4)	(90)	(75)	(10.0)	(0)

In general, MDR-TB shows equivalent clustering rate, thus, equivalent transmissibility with drug sensitive strains.

It was suggested that ST 2 and ST 4 more readily acquire the S315T mutation in *katG*.
Or ongoing transmission could be the driving force of such a high prevalence of the *katG* S315T mutation.

Clinical and demographic data

ST	No. MDR / No. DS-TB	Average Age		Ratio of Male		New Cases %	
		DS	MDR	DS	MDR	DS	MDR
2	22 / 9	54.9	54.6	70	81.8	90	18.2
3	8 / 30	58.8	59.1	73.3	75	76.7	25
4	34 / 44	57.2	58.8	75	70.6	84.1	14.7
8	10 / 9	54.2	55.8	55.6	90	100	20

High rate of re-occurrence of TB in ST 2 and ST 4 could be explained certain extent to the clonally evolution causing MDR from the original DS-TB although exogenous re-infection could not be neglected.

DS →MDR ST 2

Summary

(1)The different sublineages of Beijing genotype TB may differ in their mechanisms of adaptation to drug selective pressure.

(2)The overabundance of MDR-, XDR-TB in ST 2 and ST 4 have been observed.

(3)Both clonally evolution and ongoing transmission could be the driving force of the overabundance.

(4)It is suggested that ST 2 and ST 4 more readily acquire the S315T mutation in *katG*.

Green: XDR-TB

Distribution of Beijing TB and Drug Resistance

Different picture must be drawn by "Asian Concerted Action on Population Structure Analysis of *M. tuberculosis* Beijing Genotype".

We can get insights into:
- **Origin of Beijing genotype.**
- **Reason for global dissemination.**
- **Reason for a variation of the drug resistance patterns in different countries.**

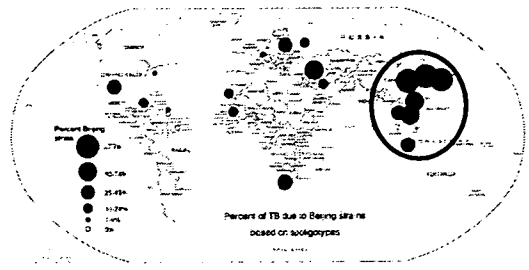
⊘ Association not known (Emerging Infectious Diseases vol. 12, 736-743, 2006)

Localization and Global Standardization of Variable Numbers of Tandem Repeat (VNTR) for TB

Takayuki Wada

Department of Microbiology,
Osaka City Institute of Public Health
and Environmental Sciences

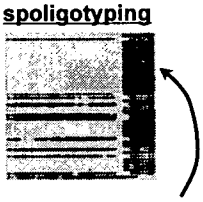
Beijing family strains have been endemic in East Asia.



Glynn, *et al.*,
Emerg Infect Dis 8:843-9.

Beijing family strains are difficult to analyze their genotypes.

- spoligotyping



Beijing strains were almost identical.

- RFLP

High discriminatory power even in Beijing family

Difficulty of comparison

Same or Different ??

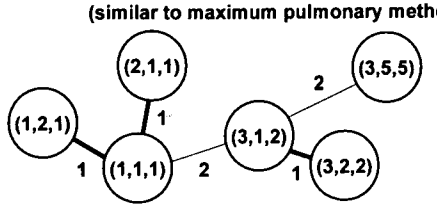
VNTR genotyping for TB

Phylogenetic tree based on VNTR by minimum spanning tree (MST)* is reliable. (1)

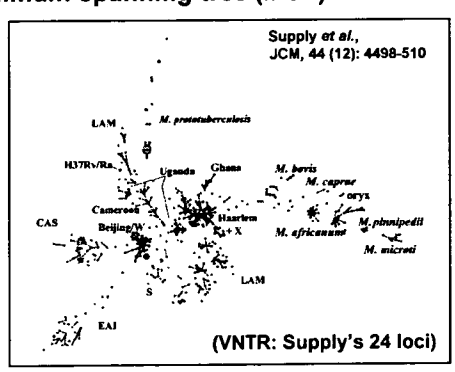
*What's MST?

- A graphic algorism to reconstruct phylogenetic trees.
- MST reconstructs a tree that connects all genetic profiles in such a way that the summed genetic distance of all branches is minimized.

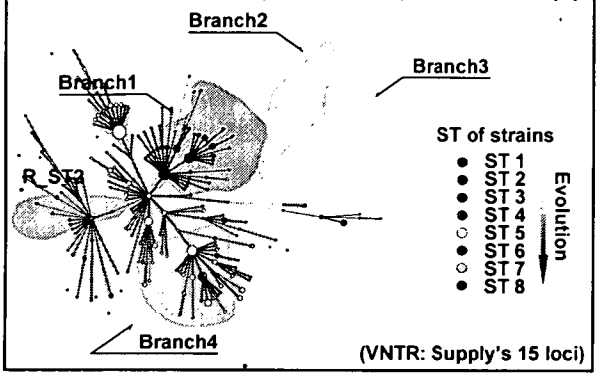
(similar to maximum pulmonary method)



Phylogenetic tree based on VNTR by minimum spanning tree (MST) is reliable. (2)



Phylogenetic analysis based on VNTR by minimum spanning tree (MST) is reliable. (3)



Standardization of VNTR loci for Beijing family strains is still confusing.

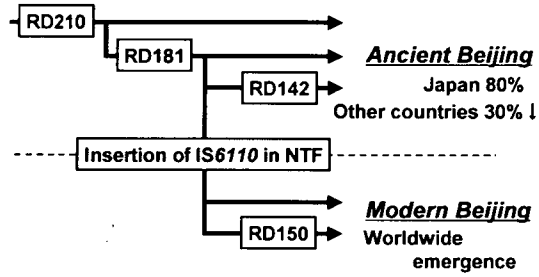
Authors	*Year*	*Area*	*Analyzed loci*
Banu et al	2004	Bangladesh	MIRU (12)
Sun et al	2004	Singapore	MIRU (12)
Mokrousov et al	2004	Russia	MIRU (12)
Kremer et al	2005	Hong Kong	MIRU (12) + ETR + HV
Surikova et al	2005	Russia	MIRU (12) + ETR + HV
Kam et al	2006	Hong Kong	ETR + HV
Nikolayevskyy et al	2006	Russia	MIRU (12) + ETR + HV
Iwamoto et al	2007	Japan (Kobe)	Supply (24) + HV
Yokoyama et al	2007	Japan (Chiba)	Supply (24) + HV
Wada et al	2007	Japan (Osaka)	MIRU (12) + ETR + HV
Millet et al	2007	Japan (Okinawa)	MIRU (12) + HV
Jiao et al	2008	China	Supply (24) + Mtub29

Localization

VS

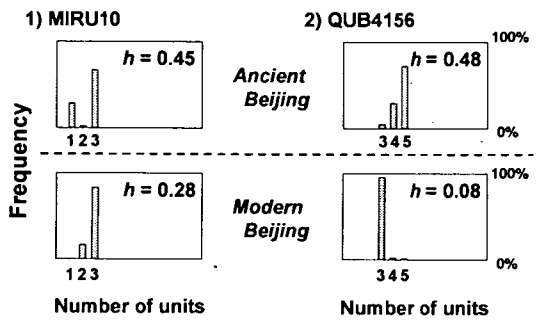
Global standardization

The population structure of Beijing family seem to be specific to each area.

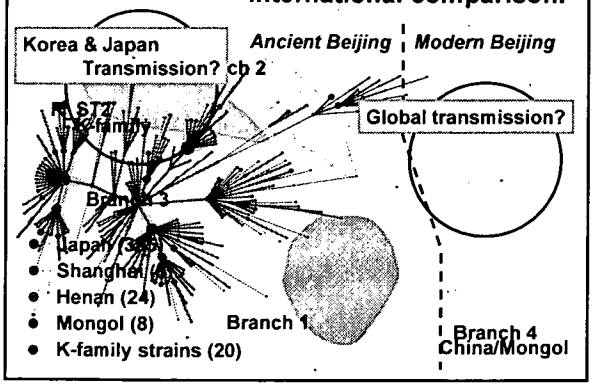


The ancient Beijing family strains has been predominant in Japan.

The specific population structure can effect the diversity of certain VNTR loci.

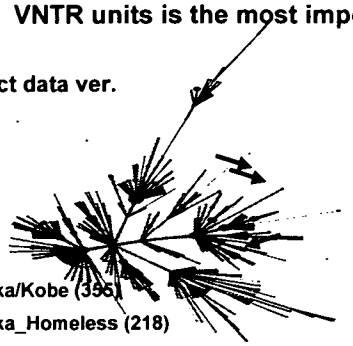


Global standardization is essential for international comparison.



The accurate calculation of VNTR units is the most important. (1)

Correct data ver.

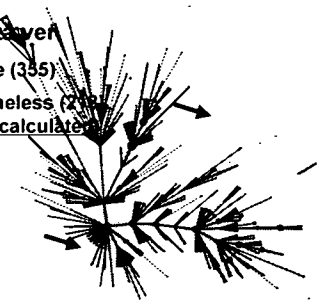


- Osaka/Kobe (356)
- Osaka_Homeless (218)

The accurate calculation of VNTR units is the most important. (2)

Incorrect data ver.

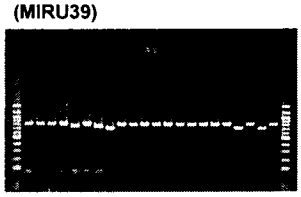
- Osaka/Kobe (355)
- Osaka_Homeless (218)
  (Mtub21 +1 calculated)



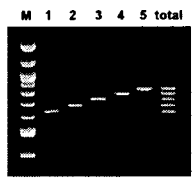
Quality control for VNTR genotyping (1)

・Agarose gel electrophoresis

(MIRU39)



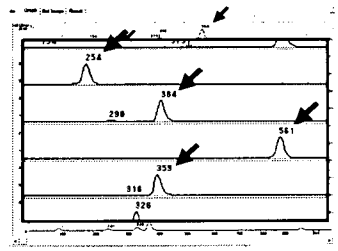
(MIRU16)



Specific markers are recommended to determine VNTR genotypes.

Quality control for VNTR genotyping (2)

・Capillary (or a sequencer) electrophoresis



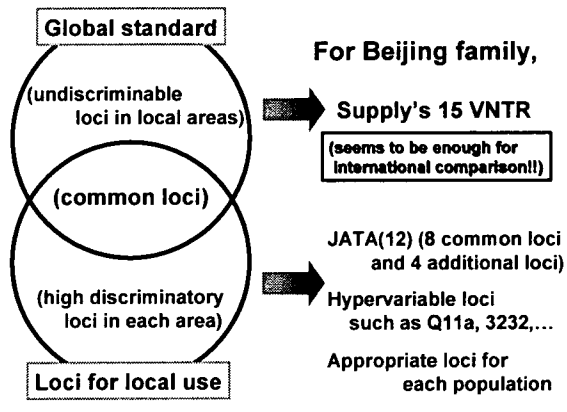
A table for calculations of number of units from the size of PCR products is useful.

International comparison and phylogenetic analysis of VNTR genotype will lead to...

1) Detection of transmission of TB isolates among countries (Epidemiology).

2) Finding specificities of genetic population structure of pathogens (Phylogeography).

3) Study for adaptive pathogenesis to the specific host population (Pathology).



2nd Meeting for researchers in charge of tuberculosis molecular epidemiology in Japan, China and Korea

(1) We show information of primers that we use

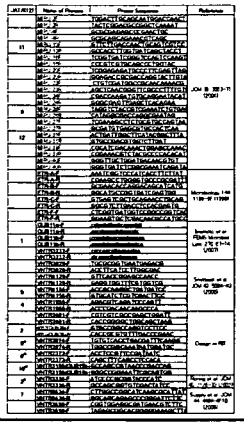
(2) Confirm the definition of copy number in each locus

(RIT provides the table of the copy number of H37Rv)

(3) We provide the 4 kinds of DNA for quality control

---

(1)We show information of primers that we use



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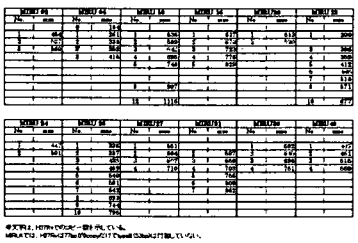
(2) Confirm the definition of copy number in each locus



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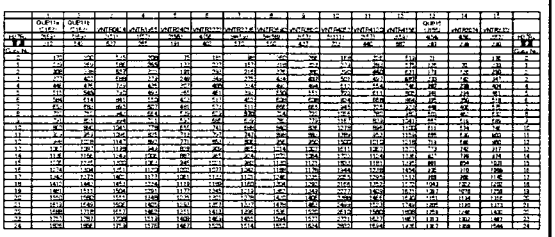
(2) Confirm the definition of copy number in each locus (2)



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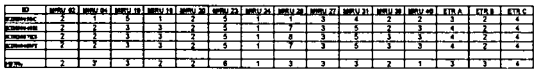
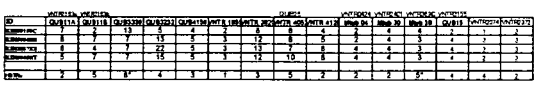
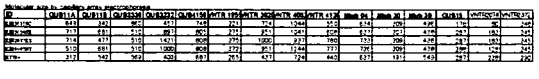
(3) We provide the 4 kinds of DNA for quality control





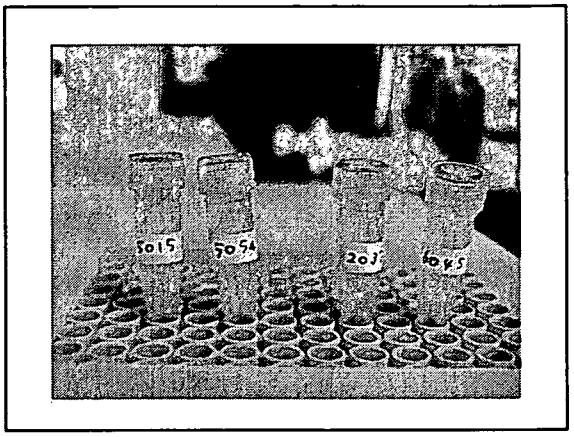


We plan to hold next meeting in Nov, 2008

Please analyze many TB isolates in for country by VNTR analyses.

19 loci :Supply (15) that reported in 2006 and 4 original loci in JATA(12)

We can analyze the phylogeny of TB in next meeting



厚生労働科学研究費補助金（新興・再興感染症研究事業）

結核菌に関する研究

薬剤耐性の迅速診断法の開発に関する研究

分担研究者：
切替　照雄　国立国際医療センター研究所　感染症制御研究部長

研究要旨

結核菌の薬剤感受性試験は数週間から数ヶ月間を要し、これに代わる迅速診断法の開発と臨床応用が急務となっている。昨年度までピラジナミド耐性遺伝子ラインプローブ法を開発した。本年度はその評価試験を進めた。また、抗結核薬の中で特に重要なイソニアジドに関して、ラインプローブ法による迅速診断法の開発を進めた。既知のイソニアジド耐性遺伝子 katG の全長と inhA のプロモーター領域を対象にしたラインプローブを試作した。このイソニアジド耐性検出率は 68%であった。そこで、検出感度のさらに高いイソニアジド耐性遺伝子検出キットの作成を目指すことを目的に、これまでの文献情報を精査して独自の解析領

A. 研究目的

本研究の目的は、昨年度までに作製したピラジナミド耐性遺伝子ラインプローブ法を臨床検体に対して試験し評価すること、ならびにイソニアジド耐性に関して、ラインプローブ法を用いた迅速遺伝子診断法を開発することである。

ピラジナミド耐性遺伝子ラインプローブ法の評価試験は、ニプロ株式会社と三菱化学メディエンス株式会社と共同で実施し、精度、感度を評価する。

抗結核薬の多くが、染色体上の比較的限定された領域への変異に起因するのに対し、イソニアジドは作用機序、耐性機序、耐性遺伝子に関して不明な点が多い。そのため、イソニアジド耐性の検出率は 60-90%と低く、改良の余地が大きい。本年度は、文献情報からイソニアジド耐性遺伝子を精査し、独自の解析領域を設定し、ラインプローブ法を用いたイソニアジド耐性検出率を向上させるための検討を推進した。

B. 研究方法

【B-1. ピラジナミド耐性遺伝子ラインプローブ法の評価試験】

平成 20 年 3 月 7 日現在、三菱化学メディエンス株式会社において、収集した 102 株に対してピラジナミド耐性遺伝子ラインプローブ法を実施した。

薬剤感受性試験については 4 月以降に実施予定である。

【B-2. イソニアジド耐性遺伝子ラインプローブ法の開発】

既知イソニアジド耐性遺伝子 katG の全長と inhA のプロモーター領域（図 1）に関して、38 のイソニアジド耐性臨床分離株を解析した。その結果、22 種類の変異を見出し、ラインプローブを作製した。しかし、検出率は 68%と低かった（図 2）。そこで、独自

の解析領域を再設定し、変異情報を蓄積することを推進した。

1. 解析領域の設定

イソニアジド耐性遺伝子に関わる論文を精査し、furA-katG オペロンとそのプロモーター領域、fabG1-inhA オペロンとそのプロモーター領域、ndh とそのプロモーター領域、ahpC とそのプロモーター領域、計 4 領域・7,157bp を解析対象に設定した（図３）。

2. 菌株の収集

国立国際医療センター、国立病院機構東京病院、結核予防会結核研究所から、臨床分離株を収集した。

3. ゲノム DNA の抽出

ゲノム DNA の抽出は、Ausubel らの方法（Current protocols in molecular biology., 1998）に従った。

4. 解析領域の増幅

4 領域の両端に特異的に結合するプライマーを設計し、PCR によって増幅した。

5. 塩基配列の決定

増幅した DNA フラグメントからプライマーを除去し、4 領域の全塩基配列を決定するために 18 種類のプライマーを設計した。サイクルシークエンス反応からダイターミネーター法による塩基配列の決定に至るまでの作業は Applied Biosystems 社の推奨方法に従った。

6. 塩基配列の解析

塩基配列の解析及び編集は、Genetyx ソフトウェアを用いて行った。M. tuberculosis H37Rv 株の塩基配列をリファレンスとし、変異の有無を調べた。

【倫理面への配慮】

研究対象は、患者情報と完全に切り離された臨床分離株である。

C. 研究結果

【C-1. ピラジナミド耐性遺伝子ラインプローブ法の評価試験】

平成 20 年 3 月 7 日現在、三菱化学メディエンス株式会社において、収集した 102 株に対してピラジナミド耐性遺伝子ラインプローブ法を実施した結果、全株がピラジナミド感受性であるという結果が出た。MGIT を用いたピラジナミド感受性試験は 4 月以降に行う予定であるため、現時点で検出率は算出できない。今後、ピラジナミド耐性菌を含め、更に 200 株を収集予定である。

【C-2. イソニアジド耐性遺伝子ラインプローブ法の開発】

本年度は、ラインプローブ作製のために独自の解析領域を設定し、変異の有無を調べることを推進した。

平成 20 年 3 月 19 日現在、109 株の臨床分離株について、解析対象の全塩基配列を決定し、変異の有無を確認した。109 株の内訳は、イソニアジド耐性株 92、イソニアジド感受性株 17 である。92 株のイソニアジド耐性菌のうち、既知変異を持っていたものは 64 株（69.6%）だった。本研究では新規変異を 22 種類見出した。新規変異だけを持つイソニアジド耐性菌は 24 株（26.1%）だった。また、新規変異の中で特に出現頻度の高いものが 2 種類あり、それぞれ 14 株（15.2%）、15 株（16.3%）で見出された。既知変異あるいは新規変異を持つ耐性菌は 88 株（95.7%）だった（表１）。

D. 考察

臨床分離株からゲノム DNA を抽出し、薬剤耐性遺伝子内の変異の有無によって、耐性化を判断することは、既報論文などからも迅速で精度の高い方法であると考えられる。特にリファンピシン耐性に対する rpoB 変異やピラジナミド耐性に対する pncA 変

異は、限定された一遺伝子内変異に耐性化の大部分が起因するため、非常に有用である。

しかし、この方法を実施するには、高価なシークエンサーと試薬が必要であり、検査室への導入が難しい。

問題点を克服するために耐性遺伝子のPCRによる増幅とラインプローブ法を組み合わせ、より安価でより迅速な遺伝子診断法を開発する。

【D-1. ピラジナミド耐性遺伝子ラインプローブ法の評価試験】

MGITを用いたピラジナミド感受性試験が未実施であるため、検出率を算出することはできなかった。順次、感受性試験を実施しつつ、ピラジナミド耐性株を含めて更に200株を収集予定である。

【C-2. イソニアジド耐性遺伝子ラインプローブ法の開発】

本年度は、ラインプローブ作製のために独自の解析領域を設定し、変異の有無を調べることを推進した。結果として、これまでに92のイソニアジド耐性菌の解析を終え、22種類の新規変異を見出した。既知変異あるいは新規変異を持つイソニアジド耐性菌は95.7%に達した。この結果が単純に検出率の向上に繋がるとは考えていない。新規変異と既知変異の一部に対する機能解析を進めることで評価していきたい。また、新規変異の中で特に出現頻度の高いものが2種類あり、それぞれ15.2%、16.3%のイソニアジド耐性菌が変異を保有していた。これら変異のどちらか一方を持つ株は30.4%にも達し、イソニアジド耐性に関わる新たなマーカーになる可能性が示唆された。
92株のうち、4株は解析領域にいかなる変異も持っていなかった。次年度はこれらの株を使って、新規イソニアジド耐性遺伝子のスクリーニング、同定、機能解析も行う予定である。

E. 結論

試作したピラジナミド耐性遺伝子ラインプローブ法の評価試験を推進した。
イソニアジド耐性遺伝子ラインプローブ法作製のために、独自の解析領域を設定し、変異情報を蓄積した。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

1) Sekiguchi J, Miyoshi-Akiyama T, Augustynowicz-Kopeć E, Zwolska Z, Kirikae F, Toyota E, Kobayashi I, Morita K, Kudo K, Kato S, Kuratsuji T, Mori T, Kirikae T. Detection of multidrug resistance in Mycobacterium tuberculosis. J Clin Microbiol 2007; 45:179-192.

2) Huang Q, Tonge PJ, Slayden RA, Kirikae T, Ojima I. FtsZ: a novel target for tuberculosis drug discovery. Curr Top Med Chem. 2007; 7:527-543.

3) Sekiguchi J, Nakamura T, Miyoshi-Akiyama T, Kirikae F, Kobayashi I, Augustynowicz-Kopec E, Zwolska Z, Morita K, Suetake T, Yoshida H, Kato S, Mori T, Kirikae T. Development and evaluation of a line probe assay for rapid identification of pncA mutations in pyrazinamide-resistant Mycobacte- rium tuberculosis strains. J Clin Microbiol 2007; 45:2802-2807.

2. 学会発表

1) Kirikae T, TB genetic mechanism of drug resistance in M. tuberculosis and novel testing techniques., US-JAPAN cooperative medical science program 12th international conference on emerging infectious diseases in the pacific

rim Antimicrobial Resistance (AMR) in respiratory infections., 2007, China.

H. 知的所有権の出願・登録状況

1. 特許出願

なし

2. 実用新案登録、その他

なし

図１：既知イソニアジド耐性遺伝子とプロモーター領域

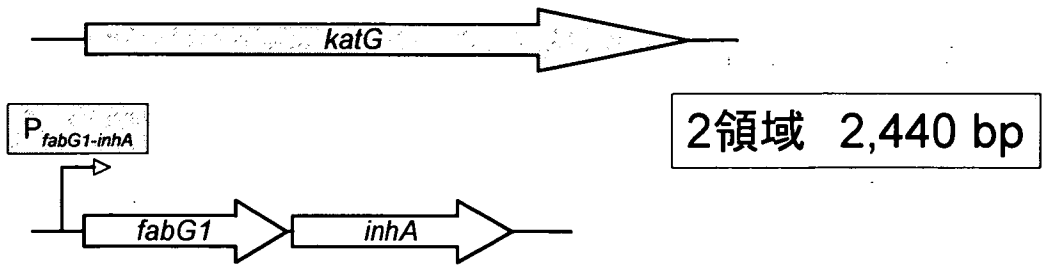
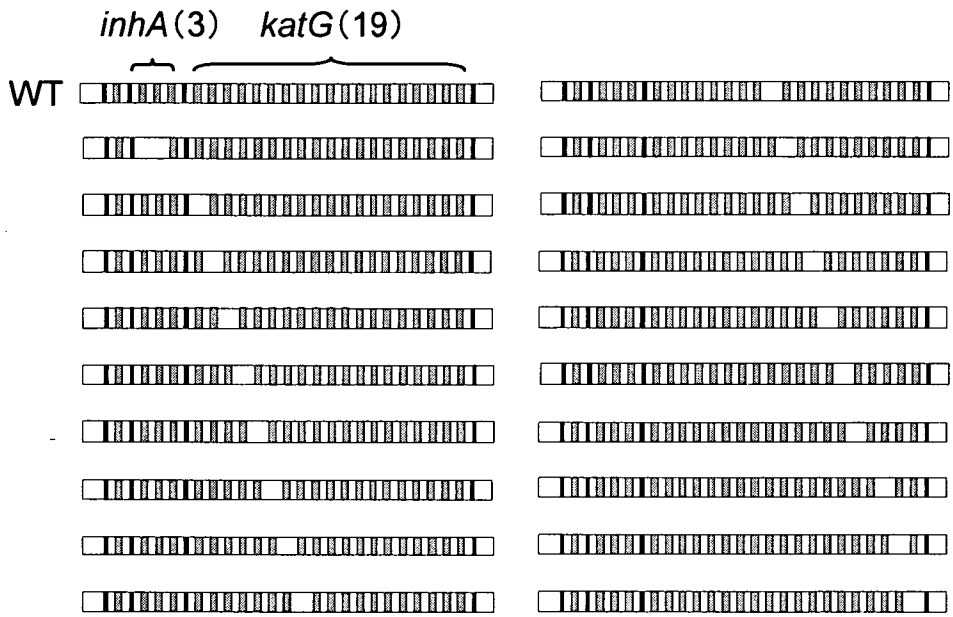


図２：試作したラインプローブの模式図と検出率



臨床試験結果：INH耐性菌検出率68%

図２：イソニアジド耐性に関する解析領域

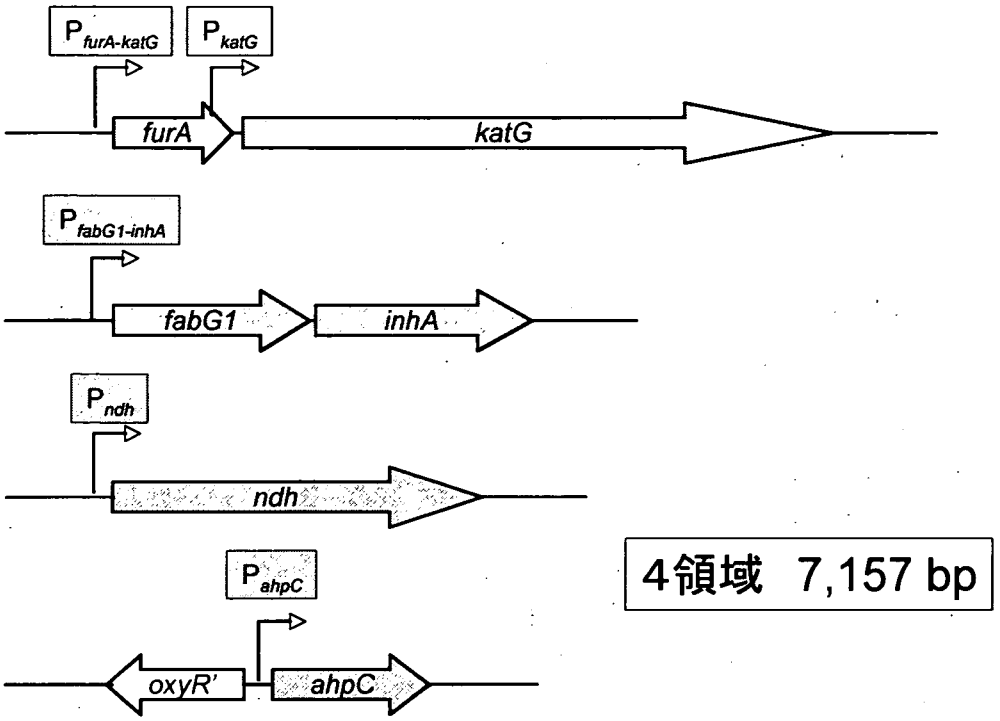


表１：解析総数と変異の内訳

解析総数	109
INH耐性株	92
INH感受性株	17

	株数	割合(%)
変異なし	4	4.3
既知変異を含む	64	69.6
既知変異のみ	45	48.9
新規変異を含む	44	47.8
新規変異のみ	24	26.1
既知変異と新規変異を含む	19	20.7
既知変異もしくは新規変異を含む	88	95.7